THROMBOTECT - a randomized study comparing low molecular weight heparin, antithrombin and unfractionated heparin for thromboprophylaxis during inudction therapy of acute lymphoblastic leukemia in children and adolescents

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THROMBOTECT – a randomized study comparing low molecular weight heparin, antithrombin and unfractionated heparin for thromboprophylaxis during induction therapy of acute lymphoblastic leukemia in children and adolescents

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Running head: Thromboembolism and thromboprophylaxis in paediatric ALL

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Abstract

Thromboembolism is a serious complication of induction therapy for childhood acute lymphoblastic leukemia. We prospectively compared efficacy and safety of antithrombotic interventions in the consecutive leukemia trials ALL-BFM 2000 and AIEOP-BFM ALL 2009. Patients with newly diagnosed acute lymphoblastic leukemia (n=949, age 1 to 18 years) were randomized to receive low-dose unfractionated heparin, prophylactic low-molecular-weight heparin (enoxaparin) or activity-adapted antithrombin throughout induction therapy. Primary objective was to test whether enoxaparin or antithrombin reduce the incidence of thromboembolism as compared to unfractionated heparin. Principal safety outcome was hemorrhage; leukemia outcome was a secondary endpoint. Thromboembolism occurred in 42 patients (4.4%). Patients assigned to unfractionated heparin had a higher risk of thromboembolism (8.0%) compared with those randomized to enoxaparin (3.5%; P=0.011) or antithrombin (1.9%; P<0.001). The proportion of patients who refused antithrombotic treatment as allocated was 3% in the unfractionated heparin or antithrombin, and 33% in the enoxaparin arm. Major hemorrhage occurred in eight patients (no differences between the groups). 5-year-event free survival was 80.9±2.2% if assigned to antithrombin compared to 85.9±2.0% in the unfractionated heparin (P=0.06), and 86.2±2.0% in the enoxaparin group (P=0.10). In conclusion, prophylactic use of antithrombin or enoxaparin significantly reduced thromboembolism. Despite the considerable number of patients rejecting the assigned treatment with subcutaneous injections, the result remains nonambiguous. Thromboprophylaxis - for the present time primarily with enoxaparin - can be recommended for children and adolescents with acute lymphoblastic leukemia during induction therapy. Whether and how antithrombin may affect leukemia outcome remains to be determined.

247 words (abstract)

Introduction

Thromboembolism (TE) is a serious complication of glucocorticoid and E. coli asparaginase-containing induction therapy for childhood acute lymphoblastic leukemia (ALL). Reported incidences vary between 1 and 37%, depending on study design and definition of thrombosis, as well as diagnostic, supportive and therapeutic methods.[1-6] Acquired antithrombin deficiency as a result of asparaginase-induced asparagine depletion is considered to be a crucial mechanism for the development of TE during ALL induction therapy. The presence of a central venous catheter (CVC) seems to be an additional – at least local – risk factor for TE as a significant proportion of TE during ALL treatment is related to an indwelling CVC. Furthermore, the risk of TE has been shown to be associated with CVC location and insertion technique.[1,5,7-12] Published data also provide good evidence for adolescent age to be an important risk factor for TE whereas the additional impact of inherited thrombophilia has been discussed controversially in the context of childhood ALL treatment.[5,13-16]

Sufficiently powered randomized trials on thromboprophylaxis in children during ALL induction therapy have not been available,[16-23] and evidence for the benefit of specific thromboprophylactic measures has therefore been lacking so far. In the absence of valid medical standards of care regarding thromboprophylaxis and the use of a CVC during ALL induction, various different approaches existed in the pediatric cancer centers in Switzerland and Germany in the early 2000s, each based on individual experiences and institutional standards. This unsatisfactory situation gave the impetus to initiate the THROMBOTECT trial, a prospective randomized study to evaluate the efficacy and safety of antithrombotic prophylaxis in children treated for ALL.

As drug administration through an indwelling CVC provides significant gain in comfort for the patients and increases the safety of therapy with tissue-toxic agents, the THROMBOTECT study was initially designed to include patients with implanted CVC from the initiation of the induction phase and was only later on also opened for patients without CVC. Two mechanisms of action to prevent TE were utilized in the two interventional arms of the trial:

inhibition of thrombin through inactivation of the coagulation factor X by the treatment with low-molecular weight heparin (LMWH) enoxaparin (Clexane™) and replacement of antithrombin by the plasma-derived antithrombin preparation Kybernin™ to compensate for asparaginase-related aquired antithrombin deficiency. Being aware of the published data of Nowak-Göttl *et al.,* that reported an almost 50% incidence of TE among ALL patients with a prothrombotic defect, and considering the additional risk factor of an indwelling CVC, a control arm without any intervention appeared difficult to justify.[15] The third arm therefore included continuous infusion of low-dose unfractionated heparin (UFH) while the CVC was in use, with the aim to locally prevent clot formation at the tip of the catheter and hereby preventing thrombotic occlusion of the indwelling CVC, without reaching relevant systemic anticoagulatory effects.[7,24-27] Therefore, the low-dose UFH was considered the control arm.

The current report presents the clinical results of the THROMBOTECT study with respect to the incidence of symptomatic TE and hemorrhage as primary efficacy and safety outcomes as well as the secondary safety outcome of leukemia-related survival.

Methods

Study Design

THROMBOTECT was an open-label, prospective, randomized, multicenter study to evaluate two different preventive antithrombotic measures during induction chemotherapy in children with ALL treated according to ALL-BFM 2000 (NCT 00430118) and AIEOP-BFM-ALL 2009 treatment protocols (NCT 01117441). THROMBOTECT was an add on-study to the ALL-BFM protocols and approved by the leading ethics committees of the Medical School Hannover, Germany, and St. Gallen, Switzerland, and by the local ethics committees of each participating site. Written informed consent was obtained from guardians and/or patients before randomization. The detailed study protocol is available online with the supplemental material.

Patient eligibility

Patients were eligible if treated on the ALL-BFM 2000 or AIEOP-BFM ALL 2009 protocol[28-30] and having a CVC inserted by day 8 of induction and remaining in place at least until day 33. The choice of the CVC and its maintenance was at the treating physician's discretion according to institutional guidelines. In August 2004, the protocol was amended to allow participation of patients without CVC. Exclusion criteria were known hemorrhagic disorders unrelated to leukemia, active gastrointestinal ulcer, previous cerebrovascular accident and/or known hypersensitivity to heparin.

Randomization and Study Treatment

After written informed consent had been given, randomization was performed by day 8 in a 1:1:1 ratio using permuted blocks of 6 patients and stratified by country and the glucocorticoid preparation (dexamethasone or prednisone) administered during induction.[29] Randomization was centrally performed by the ALL-BFM study coordination center using computer-generated random number lists. This ensured that the participating centers had no access to the allocation sequence. The assigned arm was submitted to the centre by fax.

Patients were randomly assigned to receive one of the two experimental thromboprophylactic treatments with either LMWH enoxaparin or with activity-adjusted antithrombin or to the control arm low-dose unfractionated heparin (UFH).

Thromboprophylaxis was started on day 8 and ended on day 33 of induction chemotherapy (Figure S1 in the Supplementary Appendix). The observation period covered the induction and consolidation phase (Figure S2 in the Supplementary Appendix) up to and including protocol day 64.

Patients in the enoxaparin group received Clexane™ at 80-100 IU/kg body weight once daily subcutaneously[31-34] with a target anti-Xa level not exceeding 0.4 U/l, measured 4 hours after the third or fourth injection. On days with lumbar puncture or other invasive procedures, enoxaparin was postponed until at least 4 hours after the procedure. In the case of

thrombocytopenia <30 x 10^9/L, platelet tranfusion was required or enoxaparin had to be held until platelet regeneration.

In the antithrombin group, antithrombin activity was measured every three days prior to each asparaginase administration. If activity was below the lower limit of normal of 80%, the plasma-derived antithrombin preparation Kybernin™ was substituted calculating the dose according to the formula [$antithrombin_{target\ 100\%}$ – $antithrombin_{actual}$] x kg body weight targeting at 100% AT activity.

Patients assigned to the control arm received UFH at 2 IU/kg body weight/hour as long as an infusion drip was running to locally prevent thrombotic occlusion of the indwelling CVC.[24]

Treatment with coagulation factors or anticoagulants beyond the interventions intended per protocol was not allowed unless clinically indicated. Management of TE was at the discretion of the treating physician.

Outcome Measures

Diagnosis of TE was based upon clinical suspicion and had to be confirmed by one or more suitable imaging methods within routine diagnostic work-up (Table S1 in the Supplementary Appendix). No systematic provision was made for blinding the attending physicians or radiologists to the randomization arm. Intermittent dysfunction of the CVC by a clot at the tip of the catheter was not considered a thrombotic event as long as CVC patency was restored.

The principal safety outcome was absence of bleeding complications during the study period. The definition of major and minor hemorrhage met internationally defined standards (Table S2 in the Supplementary Appendix).[35-37] Secondary safety outcomes were event-free survival (EFS) and overall survival (OS). EFS was defined as time from diagnosis to the date of last follow-up or first event. Events were resistance to therapy, leukemia relapse, secondary neoplasm or death from any cause. Failure to achieve remission due to early death or resistance was considered as event at time zero. Survival was defined as time from diagnosis to the date of last follow-up or death from any cause.

Statistical Analysis

The primary objective was to test whether antithrombotic prophylaxis with enoxaparin or antithrombin was superior to UFH. The null hypothesis was that there was no difference between enoxaparin or antithrombin versus UFH tested with one-tailed Fisher's exact test at a significance level of P=0.025 each. The main analysis was by intention-to-treat (ITT). In order to reach a power of 85% with a significance level of 0.025, 315 patients had to be randomized per group, assuming an event rate of 9% within the UFH group and 3% in the two interventional groups, respectively. If both comparisons were significantly different, the thrombosis rates in the enoxaparin and antithrombin arm had to be tested for equivalence (secondary objective). Antithrombin replacement and enoxaparin therapy would be considered equivalent if the two-sided 95% confidence interval (95%-CI) of the incidence difference did not exceed ±4%. For the equivalence test, patients were analyzed according to the given treatment (as treated).

The Kaplan-Meier method[38] was used to estimate survival rates, and differences were compared with the log-rank test.[39] Cox proportional hazards model was used in univariate and multivariate survival analyses.[40] Cumulative incidence functions for competing events were constructed by the method of Kalbfleisch and Prentice[41] and compared with the Gray's test.[42] Odds ratios were calculated to compare the risks of thromboembolic events. Except for the confirmative analyses of the primary study question, all other analyses were exploratory.

Results

Patient Characteristics

From December 1st, 2002, to December 31st, 2011, 1526 patients with ALL treated at one of the 26 study centers in Germany and Switzerland were eligible for randomization (Figure 1). Of these, 577 patients were not randomized, the vast majority because patients and/or parents refused consent to be randomized for the enoxaparin arm as they strictly did not wish to accept a daily subcutaneous injection. 949 patients (ITT population) were randomly

assigned to receive either UFH (N=312), enoxaparin (N=317) or antithrombin (N=320). Randomized and non-randomized eligible patients did not differ with respect to their initial patient characteristics (Table S3 in the Supplementary Appendix). The proportion of patients with poor response to the prednisone prephase (prednisone poor-responders) and slow treatment response as assessed by minimal residual disease was significantly higher in the group of non-randomized patients. In the ITT population, numbers and characteristics of patients were well balanced between the three randomization arms except for a slight imbalance in the age distribution with fewer children below six years in the enoxaparin group (Table 1). Patient characteristics were evenly distributed between the randomization arms as treated except for a significantly lower proportion of patients below 6 years of age in the enoxaparin arm (details provided in Table S4 in the Supplementary Appendix).

The proportion of patients who refused antithrombotic treatment as allocated was 3% in patients randomized to UFH (10/312) or antithrombin (11/320), and 33% (105/317) in those assigned to enoxaparin (Figure 1). Rejection of the enoxaparin arm was more frequent in patients below six years of age than in older patients (62/157 [39%] vs. 42/160 [27%]) with a preferential switch to UFH in the younger cohort (Table S5 in the Supplementary Appendix). Based on this finding additional exploratory analyses with respect to TE rate and leukemia-related outcomes were therefore performed, stratified by age and in the as-treated groups.

Thromboembolic Events

Among the 949 randomized patients, 42 thromboembolic events were observed (4.4%; 95%-CI 3.2 to 5.9). Of those, 20 events (47.6%) occurred in the upper, seven (16.7) in the lower deep venous system, and 13 (30.9%) in the cerebral sinus veins; two patients (4.8%) had a cerebral arterial stroke. Eight of the 42 TEE (19%) were distant to the site of the CVC. Thirty-three events occurred between treatment day 9 and 36 during induction therapy, nine events between treatment day 37 and 52 of induction consolidation.

Children below six years of age had a significantly lower risk of TE (14/512, 2.7%) than those aged 6 to 9 years (11/188, 5.9%) or 10 years and older (17/249, 6.8%; P=0.018). Other patient characteristics and features, such as gender, initial white blood cell count,

immunophenotype or treatment response did not influence the incidence of TE (data not shown).

The incidence of TE was significantly higher in patients randomized to UFH (25/312; 8.0%) than in the enoxaparin (11/317; 3.5%; P=0.011) or antithrombin group (6/320; 1.9%; P<0.001). The as-treated analysis revealed an incidence of 6.7% in the UFH group (25/372) compared to 3.2% in the enoxaparin (7/216; P=0.089) and 2.6% in the antithrombin group (9/341; P=0.013). The respective cumulative incidences are depicted in Figures 2A and B. The difference between TE incidences in the enoxaparin and antithrombin group as treated was -0.6%; the lower and upper limit of the 95%-CI were -3.5% and +2.3%, respectively (p-values for the corresponding one sided tests P=0.01 and P=0.001). Thus, antithrombin and enoxaparin were equally effective.

Exploratory as-treated analyses stratified by age (Figures 2D and F) demonstrated a significantly reduced risk of TE in patients six years of age or older when treated in one of the experimental arms compared to the control group (UFH: 18/158, 11.4%; enoxaparin: 5/120, 4.2%, P(vs. UFH)=0.001; antithrombin 4/150, 2.7%, P(vs. UFH)<0.001). No significant differences were found in patients below six years of age (UFH 7/214, 3.3%; enoxaparin 2/96, 2.1%; antithrombin 5/191, 2.6%).

For subgroup analysis by age no formal test for interaction was done. Applying Fine-Gray models with interaction terms for age older than 6 years and enoxaparin/antithrombin, the interactions are not significant. This, however, does not entirely exclude interactions since the power for such tests is low.

Hemorrhage

Eight bleeding episodes were documented among the 929 randomized patients (0.9%). Four of them occurred during induction chemotherapy under antithrombotic prophylaxis and 4 during consolidation after termination of the anticoagulants. All hemorrhages were classified as major (7 gastrointestinal, 1 cerebral). Four patients with hemorrhage were treated in the UFH group (1.1%), three in the antithrombin group (0.9%, P(vs. UFH)=1.0) and one patient in the enoxaparin group (0.5%, P(vs. UFH)=0.66).

Leukemia Outcome, Survival

Five-year probability of EFS (5y-pEFS) and cumulative incidence of relapse (5y-CIR) of the THROMBOTECT cohort were comparable with the 577 non-randomized patients (THROMBOTECT cohort: 5y-pEFS 84.3±1.2%, 5y-CIR 11.7±1.1%; non-randomized patients: 5y-pEFS 84.0±1.6%, 5y-CIR 11.8±1.4). Patients randomized to the antithrombin arm had a 5y-pEFS of 80.9±2.2% compared with those assigned to the enoxaparin (86.2±2.0%, P=0.10) or UFH arm (85.9±2.0%, P=0.06) (Figure 3A) with a Hazard ratio of 1.40 (1.02-1.92; P=0.040) for the antithrombin arm versus the remaining patients. The probability of OS at 5 years was similar in all three arms (antithrombin 89.8±1.7%, enoxaparin 90.9±1.6%, UFH 92.4±1.5%). The differences observed in the EFS were due to a higher incidence of late relapses in the antithrombin group as compared to the other groups (Figure 3C); the as-treated analyses showed no statistically significant difference between the three groups (Figure 3B and D; Hazard ratio antithombin vs. others: 1.16 [0.84-1.59]; P=0.37). Retrospective exploratory subgroup analyses revealed a higher relapse incidence of the antithrombin-treated patients within the medium risk group only (Figure S3 in the Supplementary Appendix). Multivariate Cox regression analyses on EFS were performed including risk group according to respective trial criteria, *TEL-AML1* status, initial white blood cell count, age and the THROMBOTECT arm as covariates. Hazard ratios for the antithrombin arm were 1.38 (0.99-1.91; P=0.054) for ITT and 1.19 (0.86-1.66; P=0.269) for the as-treated analysis and thus comparable with those of the univariate analyses (Table S6 in the Supplementary Appendix).

To test for a potential dose effect of antithrombin, doses given were analyzed in patients treated in the antithrombin arm. Data available for 248 of 341 patients (72.7%) did not disclose a dose-related effect on the relapse incidence (Figure S4 in the Supplementary Appendix).

Discussion

Reliable data on TE during induction therapy of childhood ALL are scarce. The only randomized interventional trial was the PARKAA trial (Prophylactic Antithrombin replacement in kids with ALL treated with L-asparaginase), designed to determine if there was a trend to efficacy and safety of antithrombin treatment but not powered to prove it.[16] To our knowledge, no other data from adequately designed and powered studies have been available so far to provide sufficient evidence that would allow valid recommendations.[4,5,9,19,20,23,43,44]

For the first time, the THROMBOTECT trial shows that prophylactic antithrombotic intervention significantly reduced TE during ALL induction therapy as compared to the control arm. Both interventions, enoxaparin and activity adapted AT substitution, were equally effective. Asparaginase induced AT deficiency is assumed to be the most important mechanism for the development of TE during ALL induction therapy.[45] As a consequence of asparagine depletion, asparaginase therapy leads to intracellular retention of a misfolded antithrombin, resulting in acquired antithrombin deficiency.[45,46] The THROMBOTECT trial demonstrated that maintaining the AT activity at 80% or higher throughout the induction phase could significantly protect patients from TE. Thus, correction of low antithrombin activity seems to be one effective way to prevent TE, this being consistent with clinical and laboratory data on antithrombin supplementation.[10,16,18,19,47]

A considerable number of patients eligible for the study were not randomized. In this group the rate of prednisone poor-responders was significantly higher than in the THROMBOTECT cohort. This may be attributed to a tendency of the doctors or parents to avoid additional burden from interventions of an add-on trial in particular on those patients with very poor response during the first days of treatment. However, patient characteristics were comparable between the three randomization groups except for a slight underrepresentation of younger patients assigned to enoxaparin. Yet, the main reason not to participate was the refusal to accept the daily subcutaneous enoxaparin injections. Not surprisingly, the proportion of patients and parents refusing the assigned enoxaparin was highest in young children. This demonstrates not only their reluctance to receive injections but also underlines

a considerable drawback in practical use, irrespective of the antithrombotic efficacy of enoxaparin.

Older age proved to be an important risk factor for TE as it has been reported earlier by others.[1,13,48] The best cut-off in our data was the age of six years. Exploratory analyses suggested that the benefit from either experimental arm was more pronounced in older patients than in young children. The significant benefit in risk reduction of TE with either intervention, enoxaparin or antithrombin, as compared to UFH, provides a convincing rationale for thromboprophylaxis in this age group. For younger children, the incidence of TE was low and comparable in all three randomization arms. The need of a thromboprophylaxis in ALL patients below 6 years of age could therefore be questioned. However, the study was not powered for subgroup analyses and the lack of statistical difference in TE incidence between the treatment groups in younger children may be due to insufficient power caused by the patient number as well as the lower TE incidence. Furthermore, in younger children TE may be missed as symptoms often are subtle. This is in line with the findings of the PARKAA study, showing that children with symptomatic TE tend to be older than those with clinically asymptomatic TE.[16] Even if clinically not diagnosed, asymptomatic TE may be associated with significant vessel occlusion.[16] This, in turn, can lead to the destruction of the vessel wall causing long term morbidity in terms of postthrombotic syndrome, likely becoming apparent years after the end of ALL therapy. Whether this applies to young patients with ALL remains unknown.[17] Future studies with sufficient statistical power are needed to ascertain if such interventions in small children are justified. Nevertheless, although the high proportion of patients who refused the allocation to the enoxaparin arm may complicate the interpretation of the results in this treatment arm, the reduction of TE in the global analysis appears to be sufficiently convincing to recommend thromboprophylaxis not only for older patients but for all age groups, all the more as hemorrhage is of no concern.

Most thrombotic events occurred between induction treatment day 9 and 36, the latter marking the start of induction consolidation. This confirms our experience that TE only rarely occurs at the time of ALL diagnosis but rather in the course of induction therapy.

Furthermore, not all centers were able to get a CVC inserted at the time of ALL diagnosis. For these reasons, thromboprophylaxis was started after the prednisone prephase on day 8 of induction therapy. The primary objective of the THROMBOTECT trial was to evaluate efficacy and safety of different prophylactic antithrombotic interventions during ALL induction therapy. Therefore, the duration of thromboprophylaxis was limited to induction therapy until day 33. Some of the thromboembolic events have occurred after the end of the induction phase. However, only a few of these patients had already started the consolidation phase when the thrombosis had been diagnosed. Factors that may have contributed to these late thromboses could be concurrent medical issues such as infections. Given the gradual development of a clot, the still asymptomatic thrombosis might have started towards the end of induction therapy and only become symptomatic in early induction consolidation. Since pegylated asparaginase is presently used more frequently - in the trial AIEOP-BFM ALL 2009, the second dose of this drug was given on day 26 of induction - late thromboses in induction consolidation might become more relevant as the use of pegylated asparaginase may lead to an extended asparagine depletion with disturbed coagulation patterns, including extended dysfunction of antithrombin. Irrespective of possible concomitant prothrombotic risk situations, the hypercoagulable state seems to remain ongoing beyond the end of induction therapy. Given the very low rate of hemorrhage it might therefore be advisable to extend thromboprophylaxis accordingly.

The open label assignement as well as the diagnosis of TE on clinical suspicion only are drawbacks of the THROMBOTECT study design. However, masking the antithrombotic intervention would have meant that all patients of all randomization groups would have been given subcutaneous injections, in the UFH and AT group containing placebo. To conduct the study as double-blinded trial with double dummy subcutaneous injections was not considered feasible in a large pediatric population.

Similar concerns apply to the primary outcome defined as TE based on clinical suspicion. The PARKAA study has shown a high incidence of clinically not recognized thromboses found by routine imaging screening.[16] To overcome observer bias, various and repeated

routine imaging screening for vessel occlusion at all possible anatomical sites would have been mandatory at predefined time points. This comprises ultrasound but also magnetic resonance imaging which, in young children, often requires general anaesthesia. In addition, for the time being the appropriate time points to look for vessel occlusions is not known and hence the possibility of missing a thrombosis at arbitrarily chosen time points would be high. Exposing the children to repeated extra anaesthesia with a questionable benefit was considered too high an extra burden. Therefore, the study design chosen was in favour of an open label treatment. Imaging was performed on clinical suspicion despite the acknowledged inherent drawbacks.

Evaluation of EFS and relapse rate within the THROMBOTECT randomization groups revealed the unexpected finding that patients randomized to the antithrombin group had a higher relapse incidence compared with the enoxaparin or UFH group. The differences were no longer obvious in the as-treated analysis being apparent in the medium risk group only. Although a causal relationship between the cumulative antithrombin dose and the relapse rate could not be established, the possibility that antithrombin substitution might affect leukemia outcome cannot be entirely excluded.

In conclusion, the THROMBOTECT study has for the first time demonstrated that activity-targeted antithrombin replacement as well as the use of enoxaparin lead to a significant risk reduction for TE during ALL induction therapy when compared with low-dose UFH. Bleeding was of no major concern. Thromboprophylaxis during induction therapy can therefore be recommended for children and adolescents with ALL. The higher incidence of late relapses in children with medium risk ALL assigned to the antithrombin group remains to be resolved and leads us at the present time to recommend primarily enoxaparin. Whether thromboprophylaxis contributes to minimize not only clinical but also silent thrombosis and by that long term morbidity in terms of postthrombotic syndrome remains to be determined. The THROMBOTECT results provide the rationale to develop new studies, both to elucidate a possible impact of antithrombin on leukemia outcome and to further determine the best practice to prevent TE during ALL induction chemotherapy. *3902 words (main text)*

Acknowledgement

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Author contribution

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Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

Author's disclosures of potential conflicts of interest

Both interventional drugs were provided free of charge by the respective pharmaceutical companies: Enoxaparin (Clexane™) by Sanofi and antithrombin (Kybernin™) by CSL Behring. Neither of the two companies was acting as a sponsor, they were not involved in the THROMBOTECT study design, neither in the collection and analysis of data nor in the content and wording of the manuscript. Neither of them had access to the THROMBOTECT data sets nor did they have information on unpublished results.

The authors declare no conflicts of interest. Information on authorship, contributions, financial and other disclosures is provided by the authors and available with the online version of this article at www.haematologica.org.

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Table 1. Patient characteristics by thromboprophylaxis group as assigned by randomization

	total (N=949) N (%)	UFH (N=312) N (%)	E (N=317) N (%)	AT (N=320) N (%)
Study				
ALL-BFM 2000	815 (85.9)	269 (86.2)	272 (85.8)	274 (85.6)
AIEOP-BFM ALL 2009	134 (14.1)	43 (13.8)	45 (14.2)	44 (13.8)
Sex				
Male	537 (56.6)	173 (55.4)	183 (57.7)	181 (56.6)
Female	412 (43.4)	139 (44.6)	133 (42.3)	139 (43.4)
Age				
1 – < 6 years	512 (54.0)	174 (55.8)	157 (49.5)	181 (56.6)
6 – < 10 years	188 (19.8)	57 (18.3)	72 (22.9)	59 (18.4)
≥ 10 years	249 (26.2)	81 (26.0)	88 (27.8)	80 (25.0)
Central venous catheter				
CVC in site	896 (94.4)	295 (94.6)	294 (93.3)	303 (95.2)
No CVC	53 (5.6)	17 (5.4)	21 (6.7)	15 (4.8)
WBC at diagnosis [x10^9/L]				
< 20	599 (63.1)	199 (63.8)	212 (66.9)	188 (58.8)
20 - < 100	249 (26.2)	83 (26.6)	76 (24.0)	90 (28.1)
100 - < 200	53 (5.6)	15 (4.8)	14 (4.4)	24 (7.4)
≥ 200	47 (5.0)	15 (4.8)	14 (4.4)	18 (5.6)
CNS status				
CNS negative	872 (91.9)	278 (89.1)	298 (94.0)	296 (92.5)
CNS positive	30 (3.2)	14 (4.4)	6 (1.9)	10 (3.1)
no information	47 (5.0)	20 (6.4)	13 (4.1)	14 (4.4)
Immunophenotype				
Non-T-ALL	827 (87.1)	264 (84.6)	298 (89.0)	281 (87.8)
T-ALL	120 (12.6)	47 (15.1)	34 (10.7)	39 (12.3)
no information	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)
Genetics				
t(12;21) / *TEL-AML1*				
negative	722 (76.1)	235 (75.3)	245 (77.3)	242 (75.6)
positive	199 (21.0)	65 (20.8)	63 (19.9)	71 (22.2)
no information	28 (3.0)	12 (3.8)	9 (2.8)	7 (2.2)
t(9;22) / *BCR-ABL*				
negative	924 (97.4)	303 (97.1)	309 (97.5)	312 (97.5)
positive	25 (2.6)	9 (2.9)	8 (2.5)	8 (2.5)
no information	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

	total (N=949) N (%)	UFH (N=312) N (%)	E (N=317) N (%)	AT (N=320) N (%)
t(4;11) / *MLL-AF4*				
negative	942 (99.3)	311 (99.7)	314 (99.1)	317 (99.1)
positive	7 (0.7)	1 (0.3)	3 (0.9)	3 (0.9)
no information	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Peripheral blast count on day 8 (Prednisone Response)				
< $1x10^9$/L (PGR)	880 (92.7)	291 (93.3)	295 (93.1)	294 (91.9)
≥ $1x10^9$/L (PPR)	65 (6.8)	19 (6.1)	22 (6.9)	24 (7.5)
no information	4 (0.4)	2 (0.6)	0 (0.0)	2 (0.6)
Risk group				
SR	301 (31.7)	97 (31.1)	101 (32.1)	101 (31.8)
MR	512 (54.0)	171 (54.8)	169 (53.7)	170 (53.5)
HR	136 (14.3)	44 (14.1)	45 (14.3)	47 (14.8)
MRD at end of induction				
negative	303 (31.9)	103 (33.0)	104 (32.8)	96 (30.0)
< 5×10^{-4}	316 (33.3)	107 (34.2)	113 (35.6)	96 (30.0)
≥ 5×10^{-3}	184 (19.4)	57 (18.3)	58 (18.3)	69 (21.6)
no information	146 (15.4)	45 (14.4)	42 (13.2)	59 (18.4)
MRD at week 12				
negative	579 (61.0)	187 (59.9)	202 (63.7)	190 (59.4)
< 5×10^{-4}	146 (15.4)	53 (17.0)	47 (14.8)	46 (14.4)
≥ 5×10^{-3}	43 (4.5)	16 (5.1)	12 (3.8)	15 (4.7)
no information	181 (19.1)	56 (17.9)	56 (17.7)	69 (21.6)
Randomized in induction in AIEOP-BFM ALL 2000*				
Randomized				
assigned to PDN	125 (13.2)	39 (12.5)	41 (12.9)	45 (14.1)
assigned to DXM	136 (14.3)	45 (14.4)	45 (14.2)	46 (14.4)
Not randomized	688 (72.5)	228 (73.1)	231 (72.9)	229 (71.6)

*For details see Figure S2 in the Supplementary Appendix and Reference Möricke, Blood (2016).[19]

Abbreviations: AT, antithrombin; CNS, central nervous system; CVC, central venous catheter; DXM, dexamethasone; E, enoxaparin; HR, high risk, MR, medium risk; MRD, minimal residual disease; PDN, prednisone; PGR, Prednisone Good-Response; PPR, Prednisone Poor-Response; SR, standard risk; UFH, unfractionated heparin; WBC, white blood cell count.

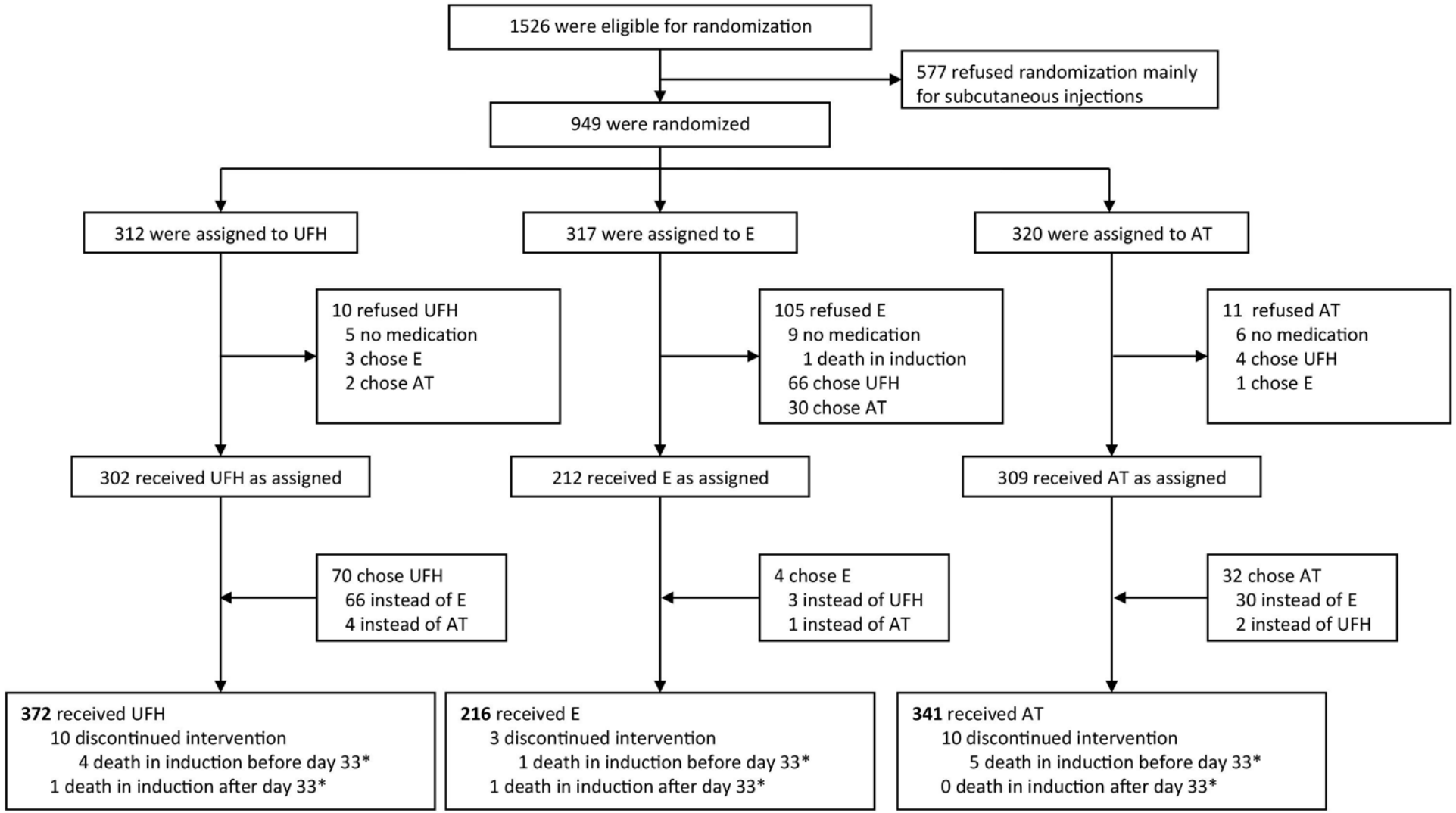
Figure legends:

Figure 1: Consolidated Standards for Reporting of Trials (CONSORT) diagram. AT denotes antithrombin, E denotes enoxaparin, UFH denotes unfractionated heparin.

Figure 2: Thromboembolic events according to the randomization arms. Results are shown by intention to treat (A, C and E) and by treatment as given (B, D and F) for the total cohort (A and B) and stratified by age < 6 years (C and D) and ≥ 6 years (E and F). Events are depicted as cumulative incidence curves. Indicated P values were calculated with the Fisher's exact test. TEE denotes thromboembolic event; UFH denotes unfractionated heparin; OR denotes Odds ratio; CI denotes confidence interval.
AT denotes antithrombin, E denotes Enoxaparin, TEE denotes thromboembolic events, UFH denotes unfractionated heparin.

Figure 3: Outcome of ALL according to the THROMBOTECT randomization arms. Event-free survival (A and B) and cumulative incidence of relapse (C and D) are shown by intention to treat (A and C) and by treatment as given (B and D). Numbers of patients at risk in the event-free survival graphs also apply to the respective relapse incidence graphs. 5 y-pEFS denotes 5-year probability of event-free survival; 5 y-CIR denotes 5-year cumulative incidence of relapse; SE denotes standard error; UFH denotes unfractionated heparin.

Figure 1



* Day 33: end of interventional treatment phase

Figure 2A

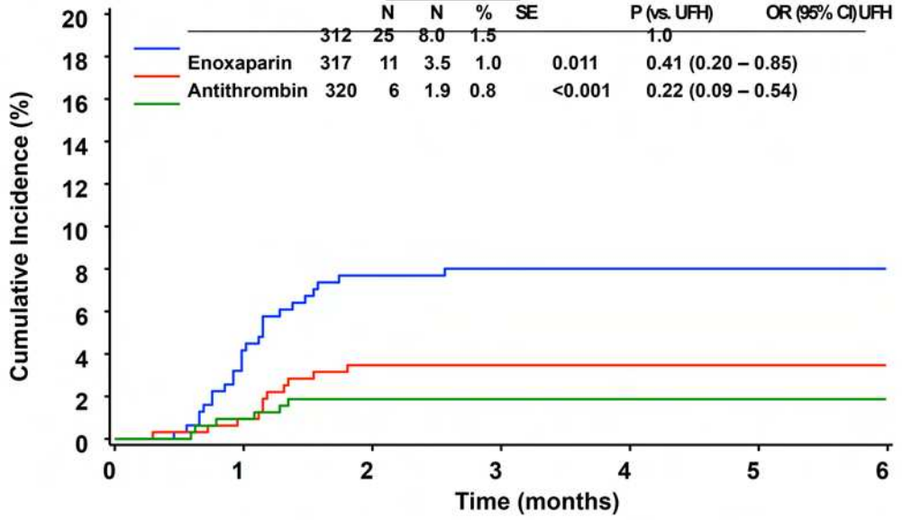


Figure 2B

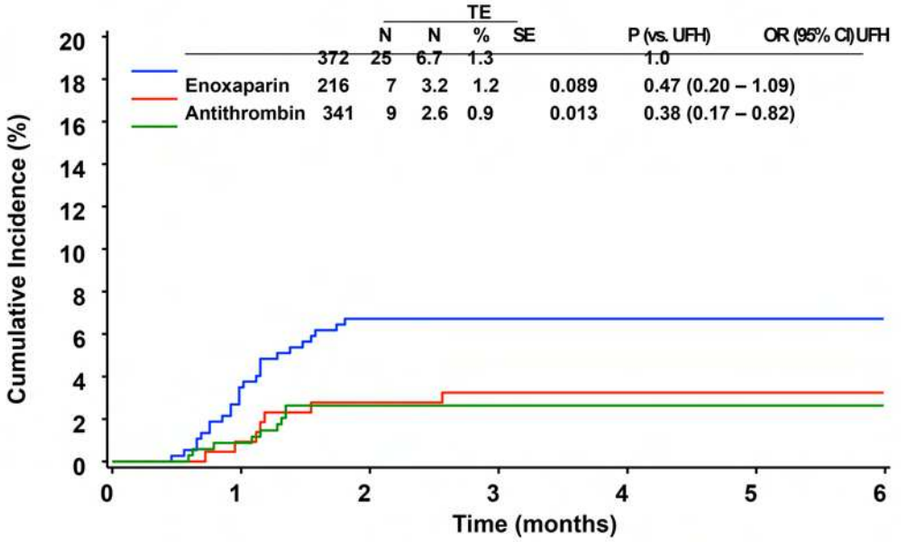


Figure 2C

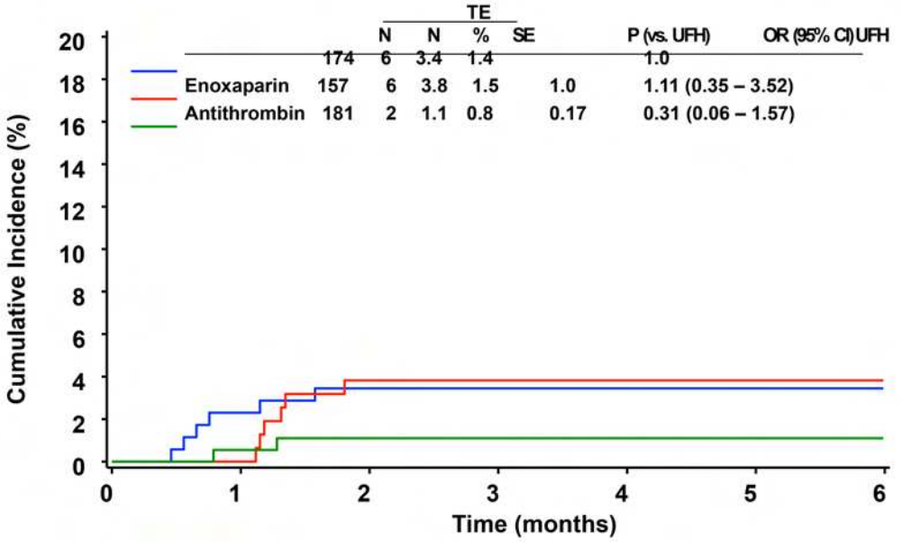


Figure 2D

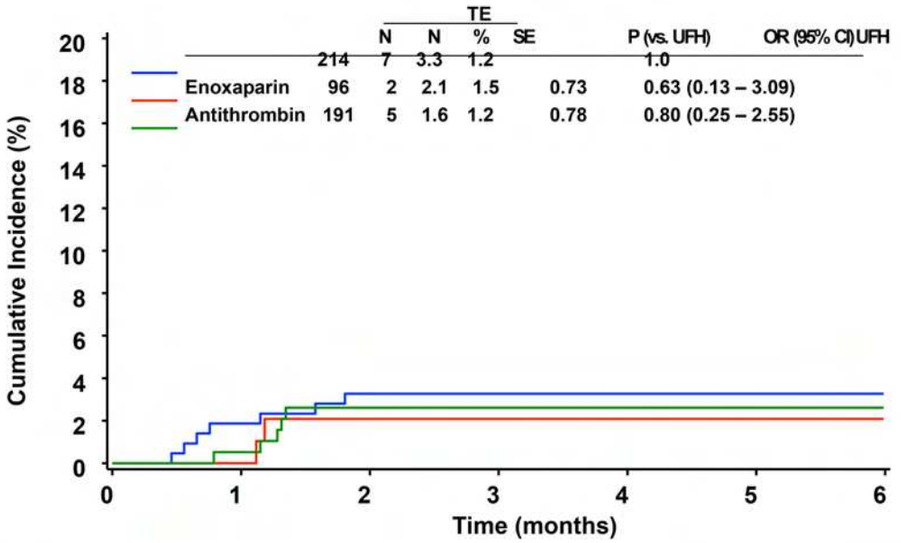
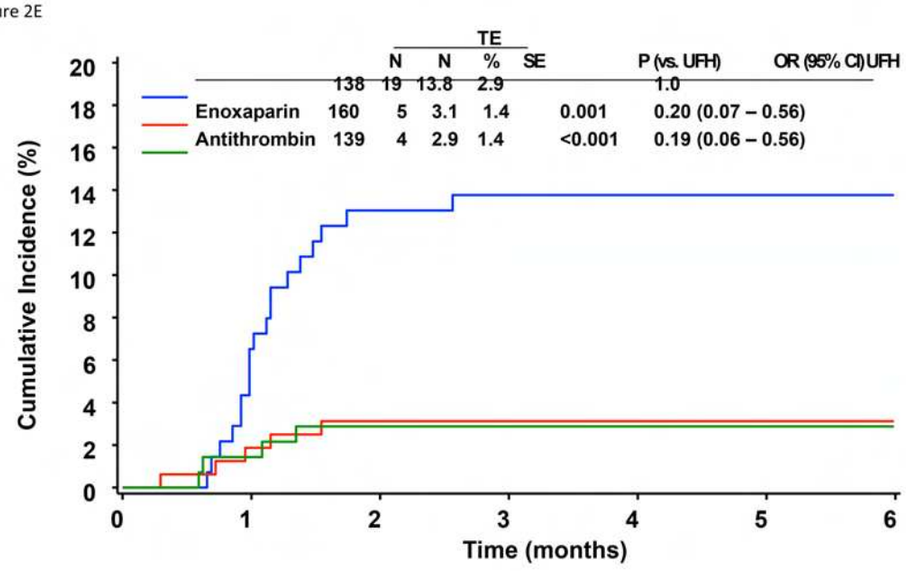


Figure 2E



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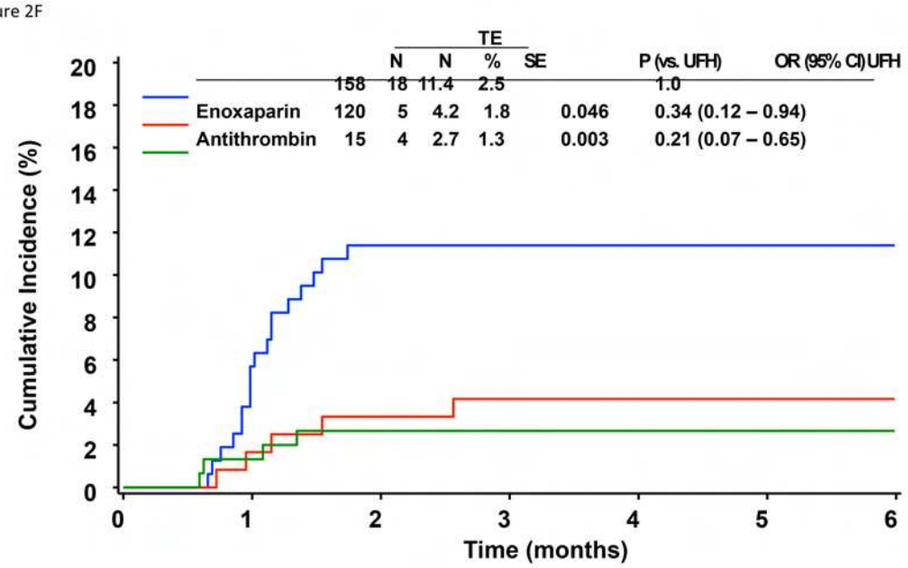
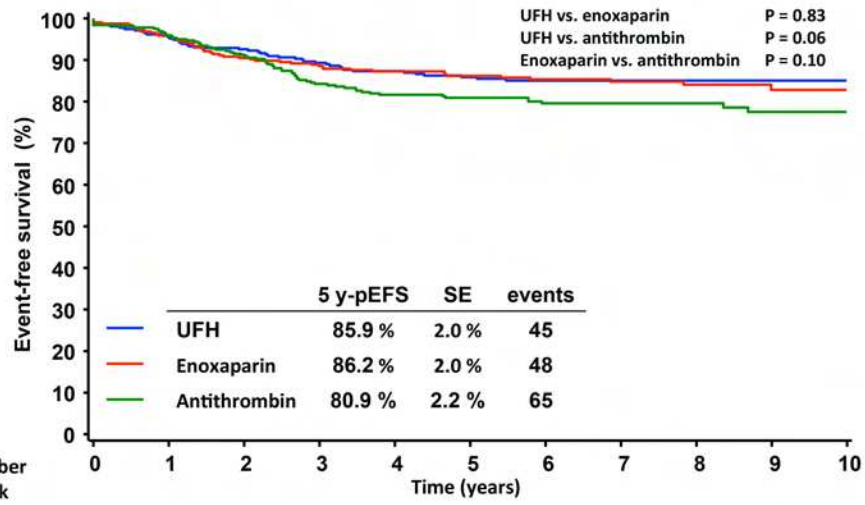
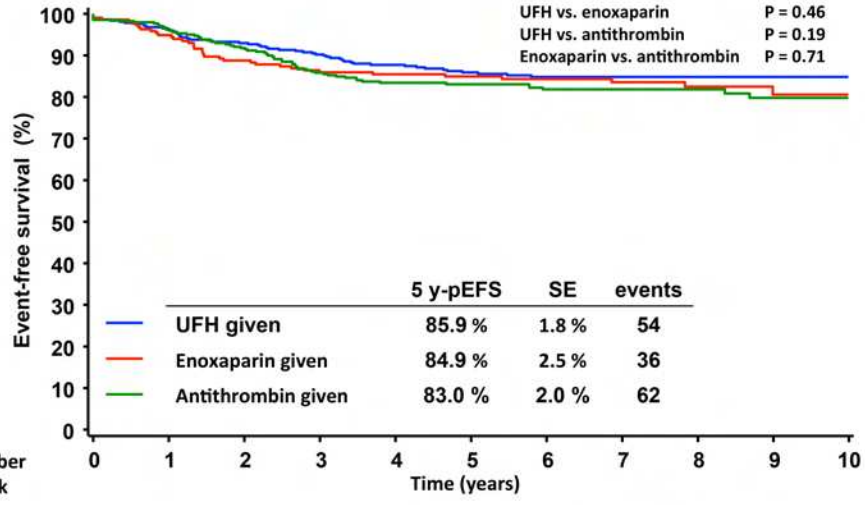


Figure 3A



Number at risk										
UFH	312	296	284	270	255	215	181	152	101	65
Enoxaparin	317	302	283	275	261	228	185	151	106	67
Antithrombin	320	306	290	264	245	213	174	124	92	64

Figure 3B



Number at risk										
UFH	372	356	340	326	307	262	214	180	122	86
Enoxaparin	216	203	189	181	175	151	127	103	73	42
Antithrombin	341	327	311	287	265	232	190	137	97	66

Figure 3C

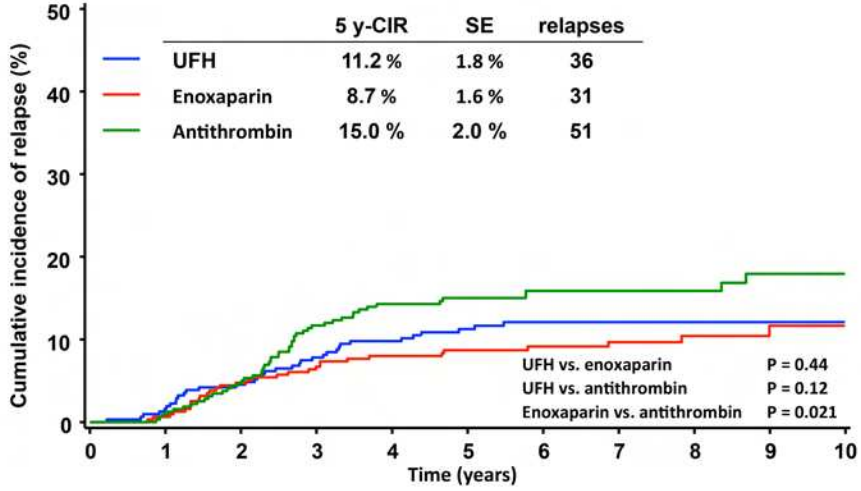
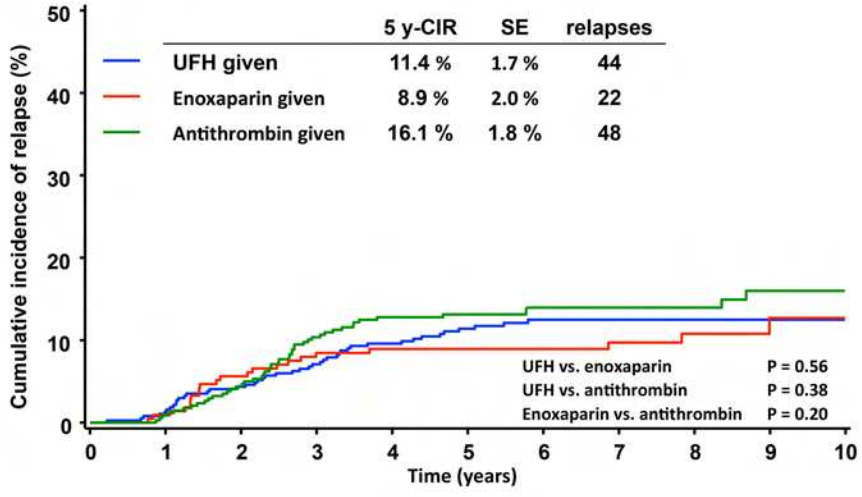


Figure 3D



Supplementary appendix to "THROMBOTECT – a randomized study comparing low molecular weight heparin, antithrombin and unfractionated heparin for thromboprophylaxis during induction therapy of acute lymphoblastic leukemia in children and adolescents"

Table of contents

List of participating centers (principal investigator; number of patients enrolled)

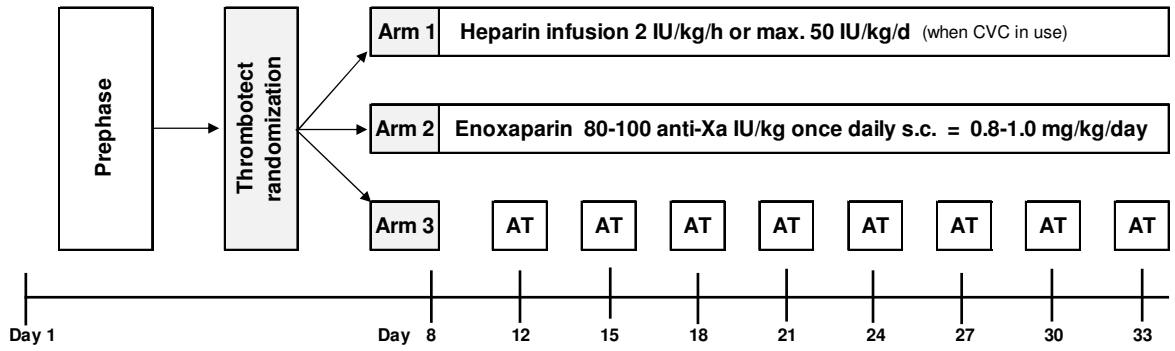
BFM Switzerland

- Luzern, Kantonsspital Luzern, Pädiatrische Klinik, Hämatologie/Onkologie (U. Caflisch, J. Rischewski; n=27)
- St. Gallen, Ostschweizer Kinderspital, Zentrum für Pädiatrische Hämatologie/Onkologie (J. Greiner; n=53)
- Zürich, Universitäts-Kinderklinik, Onkologie (F. Niggli, E. Bergsträsser, N. Bodmer; n=70)

BFM Germany

- Augsburg, I. Kinderklinik des Klinikum Augsburg, Hämatologie/Onkologie (A. Gnekow; n=69)
- Aachen, Kinderklinik der Medizinischen Fakultät der RWTH (R. Mertens, L. Lassay; n=40)
- Bad Mergentheim, Caritas Krankenhaus, Kinder- und Jugendmedizin (R. Buchhorn; n=1)
- Berlin, Charité Campus Virchow-Klinikum Berlin, Klinik für Pädiatrie mit Schwerpunkt Onkologie und Hämatologie (G. Henze, A. von Stackelberg; n=117)
- Braunschweig, Städtisches Klinikum, Klinik für Kinder- und Jugendmedizin (W. Eberl; n=26)
- Erfurt, Helios Klinikum Erfurt GmbH, Klinik für Kinder- und Jugendmedizin (A. Sauerbrey; n=30)
- Frankfurt /Main, Klinikum der Johann Wolfgang Goethe-Universität, Zentrum für Kinder- und Jugendmedizin, Klinik III, Pädiatrische Hämatologie und Onkologie (T. Klingebiel; n=29)
- Freiburg, Universitätsklinikum, Zentrum für Kinderheilkunde und Jugendmedizin, Klinik IV, Pädiatrische Hämatologie und Onkologie (C. Niemeyer; n=108)
- Hannover, Medizinische Hochschule Hannover, Kinderheilkunde IV, Klinik für Pädiatrische Hämatologie und Onkologie (K. W. Sykora, A. Beilken; n=84)
- Homburg/Saar, Universitätsklinik für Kinder- und Jugendmedizin, Pädiatrische Hämatologie und Onkologie (N. Graf, H. Reinhardt; n=24)
- Jena, Universitätsklinikum, Klinik für Kinder- und Jugendmedizin (B. Gruhn; n=1)
- Kassel, Klinikum Kassel, Klinik für Pädiatrische Onkologie/Hämatologie (M. Natrath, n=2)
- Kiel, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Klinik für Kinder- und Jugendmedizin, Pädiatrische Hämatologie und Onkologie (M. Schrappe, A. Claviez; n=70)
- Köln, Klinikum der Universität zu Köln, Kinderonkologie und –hämatologie (F. Berthold, D. Schwamborn; n=56)
- Lübeck, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Klinik für Kinder- und Jugendmedizin, Pädiatrische Hämatologie und Onkologie (P. Bucsky, M. Lauten; n=22)
- Magdeburg, Universitätsklinikum Magdeburg, Kinderklinik, Pädiatrische Hämatologie/Onkologie (U. Mittler, P. Vorwerk; n=28)
- Marburg, Universitätskinderklinik (H. Christiansen; n=2)
- Nürnberg, Cnopf'sche Kinderklinik (W. Scheurlen; n=1)
- Oldenburg, Klinikum Oldenburg GmbH, Zentrum für Kinder- und Jugendmedizin, Allgemeine Kinderheilkunde, Hämatologie/Onkologie (R. Kolb; n=69)
- Siegen, DRK-Kinderklinik (R. Burghard; n=6)
- Ulm, Universitätsklinik für Kinder- und Jugendmedizin (K. M. Debatin, C. F. Classen; n=9))
- Vechta, St. Marienhospital Vechta, Klinik für Kinder- und Jugendmedizin (J. Erkel; n=3)
- Wolfsburg, Klinikum Wolfsburg, Klinik für Kinder- und Jugendmedizin (S. Mukodzi; n=2)

Figure S1. Treatment schedule of the THROMBOTECT study



Arm 1: Unfractionated heparin 2 IU/kg/h or max. 50 IU/kg/d, when CVC is in use

- For documentation purposes, measurement of anti-Xa activity (venous blood sample, not from CVC) if infusion duration exceeds 24 h between day 16 and day 33. No dose adjustment is necessary.

Arm 2: Low molecular weight heparin (Enoxaparin) 80-100 anti-Xa units once daily s.c.

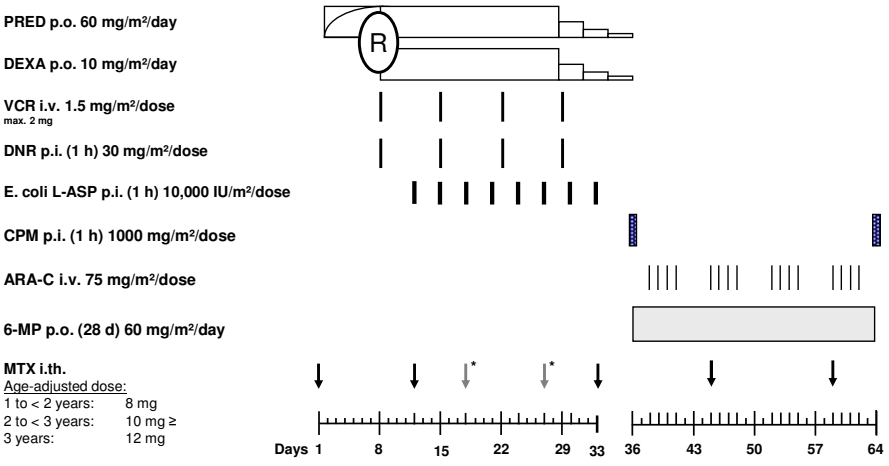
- Measurement of anti-Xa activity on day 12 if LP on day 15, or on day 15 if LP on day 12 (venous blood sample, not from CVC)
- Target value: anti-Xa activity ≤ 0.4 IU/ml [for deviations: dose adjustment of Enoxaparin and repeat measurement of anti-Xa activity after 3 days]
- no concomitant antithrombin replacement

Arm 3: Antithrombin (AT) replacement (Kybernin^{R}), if Antithrombin is < 80%

- Antithrombin measurement before each asparaginase dose (CVC may be used)
- Dosage according to the formula $AT_{nominal\ (100\%)} - AT_{actual} \times kg$
- Antithrombin can also be given on the same day, after the asparaginase

Figure S2. Treatment schedule of induction therapy in ALL-BFM 2000 and AIEOP-BFM ALL 2009

A. ALL-BFM 2000



*additional i.th. MTX on days 19 and 26 if CNS-positive (CNS 3) or presence of blasts in initial cytospin (CNS 2)

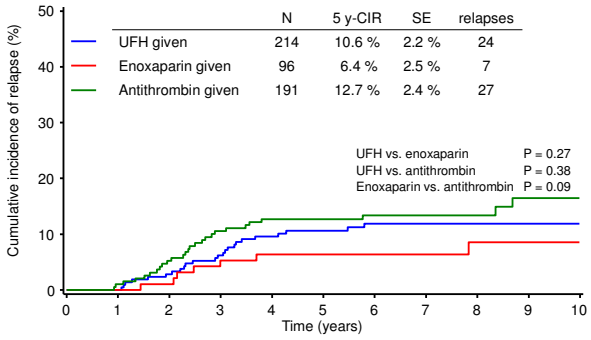
B. AIEOP-BFM ALL 2009

Induction and consolidation therapy (Protocol I) was the same as in ALL-BFM 2000 except for the following differences:

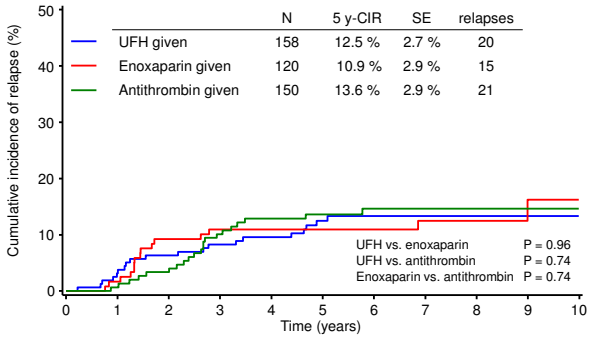
- Dexamethasone was given to good-risk T-ALL only; all other patients received Prednisone.
- Pegylated E. coli L-asparaginase given on day 12 and 26 instead of native E. coli L-asparaginase

Figure S3. Relapse incidence in specific patient subsets in analysed in randomized patients according to the treatment as given

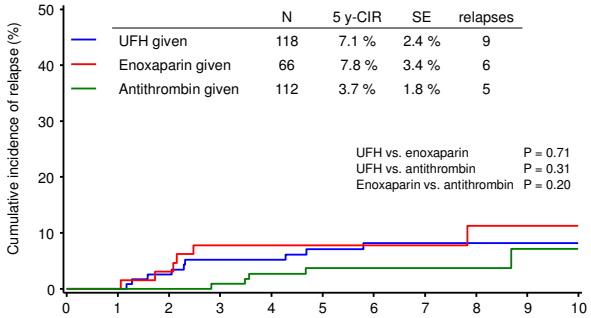
A Age < 6 years



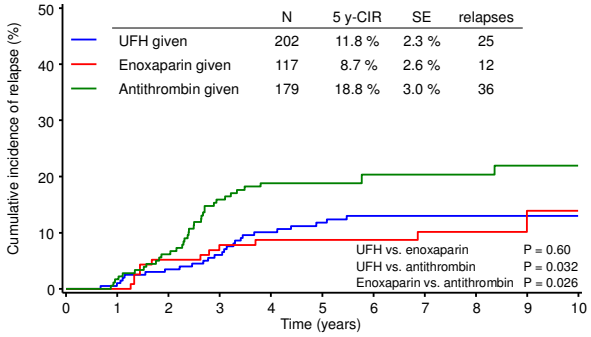
B Age ≥ 6 years



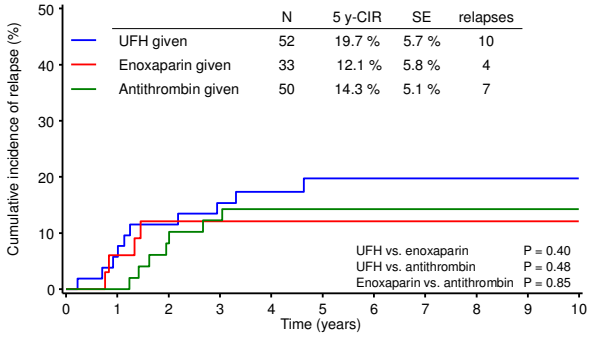
C Risk group SR (Standard Risk)



D Risk group MR (Medium Risk)



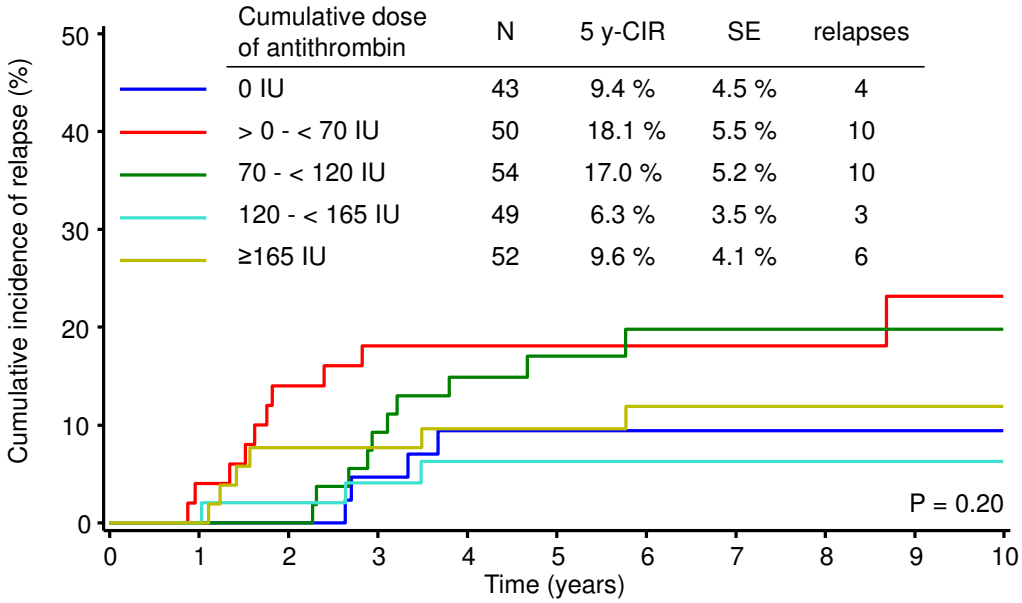
E Risk group HR (High Risk)



P values were calculated with the Gray's test.

Abbreviations: 5 y-CIR, 5-year cumulative incidence of relapse; SE, standard error; UFH, unfractionated heparin.

Figure S4. Relapse incidence of randomized patients treated in the antithrombin arm according to the cumulative antithrombin dose actually substituted per KG body weight



The P value was calculated with the Gray's test.

Abbreviations: 5 y-CIR, 5-year cumulative incidence of relapse; SE, standard error.

Table S1. Recommended diagnostic procedures for suspected thrombosis

Suspected diagnosis	Diagnostic procedures
Deep vein thrombosis	Conventional and/or pulse-wave ultrasound, and/or color Doppler ultrasound, if not conclusive ⇨ phlebography
Sinus vein thrombosis	(angio)magnetic resonance imaging
Atrial thrombosis	Echocardiography
Catheter occlusion	Imaging of the catheter tip using contrast medium (Note: identifies only thrombi at the tip of the central venous catheters (CVC), does **not** identify thrombi along the intravascular part of the catheter (sheath clot). If strong clinical suspicion of CVC-related thrombosis, perform phlebography)
Pulmonary embolism	(Ventilation) perfusion scintigraphy

Abbreviations: ALL, acute lymphoblastic leukemia

Table S2. Definition of major and minor haemorrhage [1-3]

Major hemorrhage	This category covers hemorrhages meeting one or more of the following criteria: • clinically evident • fatal • requiring erythrocyte replacement (10-20 ml/kg body weight) • hemorrhage located within the cranium/spine, or eye, or retroperitoneum (diagnosis using magnetic resonance imaging, computed tomography and/or ultrasound) • severe or life-threatening event resulting from the hemorrhage, therefore requiring intensive care
Minor hemorrhage	This category covers hemorrhages which, although clinically evident, do not meet the criteria for a major hemorrhage. Minor hemorrhages includes: • epistaxis (irrespective of platelet count) lasting more than 5 minutes, whether or not treatment is necessary • nonmechanical hematuria (i.e. not caused by urinary catheter, nephrolithiasis) • nonmechanical hemorrhages in the gastrointestinal tract (i.e. not caused by gastric tube, endoscopy, intubation) • hemorrhage of the skin and mucous membranes • subconjunctival hemorrhage • wound hematoma or minor bleeding from a new wound, as they do not satisfy the criteria for a major hemorrhage

Table S3. Patient characteristics of randomized versus eligible non-randomized patients

	randomized patients (N=949) N (%)	patients not randomized (N=577) N (%)	P (Fisher's exact)
Study			
ALL-BFM 2000	815 (85.9)	412 (71.4)	< 0.001
AIEOP-BFM ALL 2009	134 (14.1)	165 (28.6)	
Sex			
Male	537 (56.6)	335 (58.1)	0.59
Female	412 (43.4)	242 (41.9)	
Age			
1 – < 6 years	512 (54.0)	311 (53.9)	0.99
6 – < 10 years	188 (19.8)	113 (19.6)	
≥ 10 years	249 (26.2)	153 (26.5)	
WBC at diagnosis [x10^9/L]			
< 20	599 (63.1)	344 (63.4)	0.21
20 - < 100	249 (26.2)	151 (25.6)	
100 - < 200	53 (5.6)	41 (6.0)	
≥ 200	47 (5.0)	40 (5.0)	
No information	1 (0.1)	1 (0.2)	
CNS status			
CNS negative	872 (91.9)	519 (89.9)	0.88
CNS positive	30 (3.2)	19 (3.3)	
no information	47 (5.0)	39 (5.0)	
Immunophenotype			
Non-T-ALL	827 (87.1)	491 (85.1)	0.22
T-ALL	120 (12.6)	86 (14.9)	
no information	2 (0.2)	0 (0.0)	
Genetics			
t(12;21) / *TEL-AML1*			
negative	722 (76.1)	455 (78.9)	0.36
positive	199 (21.0)	110 (19.1)	
no information	28 (3.0)	12 (2.1)	
t(9;22) / *BCR-ABL*			
negative	924 (97.4)	559 (99.3)	0.63
positive	25 (2.6)	18 (0.7)	
no information	0 (0.0)	0 (0.0)	
t(4;11) / *MLL-AF4*			
negative	942 (99.3)	573 (99.3)	1.0
positive	7 (0.7)	4 (0.7)	
no information	0 (0.0)	0 (0.0)	
Peripheral blast count on day 8 (Prednisone Response)			
< 1x10^9/L (PGR)	880 (92.7)	500 (86.7)	< 0.001
≥ 1x10^9/L (PPR)	65 (6.8)	71 (12.3)	

	randomized patients (N=949) N (%)	patients not randomized (N=577) N (%)	P (Fisher's exact)
no information	4 (0.4)	6 (1.0)	
Risk group according to ALL-BFM 2000 criteria			
SR	301 (31.7)	178 (30.8)	0.003
MR	512 (54.0)	278 (48.2)	
HR	136 (14.3)	121 (21.0)	
MRD at end of induction			
negative	303 (31.9)	140 (24.3)	0.017
$< 5 \times 10^{-4}$	316 (33.3)	201 (34.8)	
$\geq 5 \times 10^{-3}$	184 (19.4)	126 (21.8)	
no information	146 (15.4)	110 (19.1)	
MRD at week 12			
Negative	579 (61.0)	278 (48.2)	0.013
$< 5 \times 10^{-4}$	146 (15.4)	98 (17.0)	
$\geq 5 \times 10^{-3}$	43 (4.5)	35 (6.1)	
no information	181 (19.1)	166 (28.8)	

Abbreviations: AT, antithrombin; CNS, central nervous system; HR, high risk, MR, medium risk; MRD, minimal residual disease; PGR, Prednisone Good-Response; PPR, Prednisone Poor-Response; SR, standard risk; WBC, white blood cell count.

Table S4. Patient characteristics by thromboprophylaxis group as treated

	total N (%)	UFH N (%)	E N (%)	AT N (%)	no treatm. N (%)
all	949	372	216	341	20
Study					
ALL-BFM 2000	815 (85.9)	321 (86.3)	188 (87.0)	290 (85.0)	16 (80.0)
AIEOP-BFM ALL 2009	134 (14.1)	51 (13.7)	28 (13.0)	51 (15.0)	4 (20.0)
Sex					
Male	537 (56.6)	202 (54.3)	136 (63.3)	189 (55.4)	10 (50.0)
Female	412 (43.4)	170 (45.7)	80 (37.0)	152 (44.6)	10 (50.0)
Age					
1 – < 6 years	512 (54.0)	214 (57.8)	96 (44.4)	191 (56.0)	11 (55.0)
6 – < 10 years	188 (19.8)	68 (18.3)	53 (24.5)	65 (19.1)	2 (10.0)
≥ 10 years	249 (26.2)	90 (24.2)	67 (31.0)	85 (24.9)	7 (35.0)
Central venous catheter					
CVC in site	896 (94.4)	355 (95.4)	200 (92.6)	327 (95.9)	14 (70.0)
No CVC	53 (5.6)	17 (4.6)	16 (7.4)	14 (4.1)	6 (30.0)
WBC at diagnosis [x10^9/L]					
< 20	599 (63.1)	247 (66.4)	135 (62.5)	205 (60.1)	12 (60.0)
20 - < 100	249 (26.2)	94 (25.3)	57 (26.4)	93 (27.3)	5 (25.0)
100 - < 200	53 (5.6)	16 (4.3)	10 (4.6)	24 (7.0)	3 (15.0)
≥ 200	47 (5.0)	15 (4.0)	13 (6.0)	19 (5.6)	0 (0.0)
CNS status					
CNS negative	872 (91.9)	337 (90.6)	203 (94.0)	315 (92.4)	17 (85.0)
CNS positive	30 (3.2)	14 (3.8)	5 (2.3)	10 (2.9)	1 (5.0)
no information	47 (5.0)	21 (5.6)	8 (3.7)	16 (4.7)	2 (10.0)
Immunophenotype					
Non-T-ALL	827 (87.1)	321 (86.3)	189 (87.5)	301 (88.3)	16 (80.0)
T-ALL	120 (12.6)	49 (13.2)	27 (12.5)	40 (11.7)	4 (20.0)
no information	2 (0.2)	2 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
Genetics					
t(12;21) / *TEL-AML1*					
negative	722 (76.1)	279 (75.0)	170 (78.7)	258 (75.7)	15 (75.0)
positive	199 (21.0)	78 (21.0)	40 (18.5)	76 (22.3)	5 (25.0)
no information	28 (3.0)	0 (0.0)	15 (4.0)	6 (2.8)	7 (2.1)
t(9;22) / *BCR-ABL*					
negative	924 (97.4)	361 (97.0)	210 (97.2)	333 (97.7)	20 (100.0)
positive	25 (2.6)	11 (3.0)	6 (2.8)	8 (2.3)	0 (0.0)
no information	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
t(4;11) / *MLL-AF4*					
negative	942 (99.3)	370 (99.5)	215 (99.5)	338 (99.1)	19 (95.0)
positive	7 (0.7)	2 (0.5)	1 (0.5)	3 (0.9)	1 (5.0)
no information	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Peripheral blast count on day 8 (Prednisone Response)					
< 1x10^9/L (PGR)	880 (92.7)	349 (93.8)	198 (91.7)	314 (92.1)	19 (95.0)
≥ 1x10^9/L (PPR)	65 (6.8)	21 (5.6)	18 (8.3)	25 (7.3)	1 (5.0)
no information	4 (0.4)	2 (0.5)	0 (0.0)	2 (0.6)	0 (0.0)
Risk group					
SR	301 (31.7)	118 (31.7)	66 (30.6)	112 (32.8)	5 (25.0)
MR	512 (54.0)	202 (54.3)	117 (54.2)	180 (52.8)	13 (65.0)
HR	136 (14.3)	52 (14.0)	33 (15.3)	49 (14.4)	2 (10.0)
MRD at end of induction					
negative	303 (31.9)	127 (34.1)	66 (30.6)	103 (30.2)	7 (35.0)
< 5 x 10^{-4}	316 (33.3)	126 (33.9)	81 (37.5)	103 (30.2)	6 (30.0)
≥ 5 x 10^{-3}	184 (19.4)	67 (18.0)	41 (19.0)	72 (21.2)	4(20.0)
no information	146 (15.4)	52 (14.0)	28 (13.0)	63 (18.5)	3 (15.0)

	total N (%)	**UFH N (%)**	**E N (%)**	**AT N (%)**	**no treatm. N (%)**
MRD at week 12					
Negative	579 (61.0)	228 (61.3)	136 (63.0)	206 (59.5)	12 (60.0)
$< 5 \times 10^{-4}$	146 (15.4)	61 (16.4)	35 (16.2)	48 (14.1)	2 (10.0)
$\geq 5 \times 10^{-3}$	43 (4.5)	18 (4.8)	9 (4.2)	16 (4.7)	0 (0.0)
no information	181 (19.1)	65 (17.5)	36 (16.7)	74 (21.7)	0 (0.0)
Randomized in induction in AIEOP-BFM ALL 2000*					
Randomized					
assigned to PDN	125 (13.2)	54 (14.5)	22 (10.2)	47 (13.8)	2 (10.0)
assigned to DXM	136 (14.3)	58 (15.6)	31 (14.4)	44 (12.9)	3 (15.0)
Not randomized	688 (72.5)	260 (69.9)	163 (75.5)	250 (73.3)	15 (75.0)

*For details see Fig S2 in the Supplementary Appendix and Reference Möricke, Blood (2016).[19]

Abbreviations: AT, antithrombin; CNS, central nervous system; CVC, central venous catheter; DXM, dexamethasone; E, enoxaparin; HR, high risk, MR, medium risk; MRD, minimal residual disease; PDN, prednisone; PGR, Prednisone Good-Response; PPR, Prednisone Poor-Response; SR, standard risk; UFH, unfractionated heparin; WBC, white blood cell count.

Table S5. Randomly assigned versus given treatment with respect to age groups

		Arm as treated			
	All patients N (%)	UFH N (%)	Enoxaparin N (%)	Antithrombin N (%)	No treatment N (%)
Age < 6 years					
All patients	512 (100.0)	214 (41.8)	96 (18.8)	191 (37.3)	11 (2.1)
Arm as assigned					
UFH	174 (100.0)	169 (97.1)	0 (0.0)	2 (1.1)	3 (1.7)
Enoxaparin	157 (100.0)	43 (27.4)	95 (60.5)	14 (8.9)	5 (3.2)
Antithrombin	181 (100.0)	2 (1.1)	1 (0.6)	175 (96.7)	3 (2.1)
Age ≥ 6 years					
All patients	437 (100.0)	158 (36.2)	120 (27.5)	150 (34.3)	9 (2.1)
Arm as assigned					
UFH	138 (100.0)	133 (96.4)	3 (2.2)	0 (0.0)	2 (1.4)
Enoxaparin	160 (100.0)	23 (14.4)	117 (73.1)	16 (10.0)	4 (2.5)
Antithrombin	139 (100.0)	2 (1.4)	0 (0.0)	134 (96.4)	3 (2.2)

Abbreviations: UFH, unfractionated heparin

Table S6. Multivariate Cox regression analyses on leukemia-related event-free survival in the randomization groups by intention to treat and as treated

	THROMBOTECT arms by intention to treat				THROMBOTECT arms as treated			
	N (%)	**Hazard ratio**	**95% CI**	***P***	**N (%)**	**Hazard ratio**	**95% CI**	***P***
Risk group								
SR	293 (31.9)	0.44	0.28-0.71	0.001	288 (32.0)	0.43	0.27-0.70	0.001
MR	496 (53.9)	1			483 (53.7)	1		
HR	131 (14.2)	1.62	1.10-2.40	0.015	129 (14.3)	1.54	1.03-2.30	0.034
TEL-AML1								
negative	721 (78.4)	1			706 (78.4)	1		
positive	199 (21.6)	0.54	0.32-0.93	0.026	194 (21.6)	0.57	0.34-0.99	0.044
WBC (x10^9/L)								
< 50	718 (78.0)	1			702 (78.0)	1		
≥ 50	202 (22.0)	1.30	0.91-1.86	0.146	198 (22.0)	1.38	0.96-1.97	0.082
Age								
< 6 years	495 (53.8)	1			484 (53.8)	1		
6 - < 10 years	183 (19.9)	0.80	0.50-1.28	0.351	181 (20.1)	0.85	0.53-1.36	0.500
≥ 10 years	242 (26.3)	1.31	0.91-1.89	0.147	235 (26.1)	1.36	0.93-1.98	0.110
THROMBOTECT arm								
UFH/enoxaparin	607 (66.0)	1			566 (62.9)	1		
antithrombin	313 (34.0)	1.38	0.99-1.91	0.054	334 (37.1)	1.19	0.86-1.66	0.296

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THROMBOTECT – a randomized study comparing low molecular weight heparin, antithrombin and unfractionated heparin for thromboprophylaxis during induction therapy of acute lymphoblastic leukemia in children and adolescents

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Running head: Thromboembolism and thromboprophylaxis in paediatric ALL

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Abstract

Thromboembolism is a serious complication of induction therapy for childhood acute lymphoblastic leukemia. We prospectively compared efficacy and safety of antithrombotic interventions in the consecutive leukemia trials ALL-BFM 2000 and AIEOP-BFM ALL 2009. Patients with newly diagnosed acute lymphoblastic leukemia (n=949, age 1 to 18 years) were randomized to receive low-dose unfractionated heparin, prophylactic low-molecular-weight heparin (enoxaparin) or activity-adapted antithrombin throughout induction therapy. Primary objective was to test whether enoxaparin or antithrombin reduce the incidence of thromboembolism as compared to unfractionated heparin. Principal safety outcome was hemorrhage; leukemia outcome was a secondary endpoint. Thromboembolism occurred in 42 patients (4.4%). Patients assigned to unfractionated heparin had a higher risk of thromboembolism (8.0%) compared with those randomized to enoxaparin (3.5%; P=0.011) or antithrombin (1.9%; P<0.001). The proportion of patients who refused antithrombotic treatment as allocated was 3% in the unfractionated heparin or antithrombin, and 33% in the enoxaparin arm. Major hemorrhage occurred in eight patients (no differences between the groups). 5-year-event free survival was 80.9±2.2% if assigned to antithrombin compared to 85.9±2.0% in the unfractionated heparin (P=0.06), and 86.2±2.0% in the enoxaparin group (P=0.10). In conclusion, prophylactic use of antithrombin or enoxaparin significantly reduced thromboembolism. Despite the considerable number of patients rejecting the assigned treatment with subcutaneous injections, the result remains nonambiguous. Thromboprophylaxis - for the present time primarily with enoxaparin - can be recommended for children and adolescents with acute lymphoblastic leukemia during induction therapy. Whether and how antithrombin may affect leukemia outcome remains to be determined.

247 words (abstract)

Introduction

Thromboembolism (TE) is a serious complication of glucocorticoid and E. coli asparaginase-containing induction therapy for childhood acute lymphoblastic leukemia (ALL). Reported incidences vary between 1 and 37%, depending on study design and definition of thrombosis, as well as diagnostic, supportive and therapeutic methods.[1-6] Acquired antithrombin deficiency as a result of asparaginase-induced asparagine depletion is considered to be a crucial mechanism for the development of TE during ALL induction therapy. The presence of a central venous catheter (CVC) seems to be an additional – at least local – risk factor for TE as a significant proportion of TE during ALL treatment is related to an indwelling CVC. Furthermore, the risk of TE has been shown to be associated with CVC location and insertion technique.[1,5,7-12] Published data also provide good evidence for adolescent age to be an important risk factor for TE whereas the additional impact of inherited thrombophilia has been discussed controversially in the context of childhood ALL treatment.[5,13-16]

Sufficiently powered randomized trials on thromboprophylaxis in children during ALL induction therapy have not been available,[16-23] and evidence for the benefit of specific thromboprophylactic measures has therefore been lacking so far. In the absence of valid medical standards of care regarding thromboprophylaxis and the use of a CVC during ALL induction, various different approaches existed in the pediatric cancer centers in Switzerland and Germany in the early 2000s, each based on individual experiences and institutional standards. This unsatisfactory situation gave the impetus to initiate the THROMBOTECT trial, a prospective randomized study to evaluate the efficacy and safety of antithrombotic prophylaxis in children treated for ALL.

As drug administration through an indwelling CVC provides significant gain in comfort for the patients and increases the safety of therapy with tissue-toxic agents, the THROMBOTECT study was initially designed to include patients with implanted CVC from the initiation of the induction phase and was only later on also opened for patients without CVC. Two mechanisms of action to prevent TE were utilized in the two interventional arms of the trial:

inhibition of thrombin through inactivation of the coagulation factor X by the treatment with low-molecular weight heparin (LMWH) enoxaparin (Clexane™) and replacement of antithrombin by the plasma-derived antithrombin preparation Kybernin™ to compensate for asparaginase-related aquired antithrombin deficiency. Being aware of the published data of Nowak-Göttl *et al.,* that reported an almost 50% incidence of TE among ALL patients with a prothrombotic defect, and considering the additional risk factor of an indwelling CVC, a control arm without any intervention appeared difficult to justify.[15] The third arm therefore included continuous infusion of low-dose unfractionated heparin (UFH) while the CVC was in use, with the aim to locally prevent clot formation at the tip of the catheter and hereby preventing thrombotic occlusion of the indwelling CVC, without reaching relevant systemic anticoagulatory effects.[7,24-27] Therefore, the low-dose UFH was considered the control arm.

The current report presents the clinical results of the THROMBOTECT study with respect to the incidence of symptomatic TE and hemorrhage as primary efficacy and safety outcomes as well as the secondary safety outcome of leukemia-related survival.

Methods

Study Design

THROMBOTECT was an open-label, prospective, randomized, multicenter study to evaluate two different preventive antithrombotic measures during induction chemotherapy in children with ALL treated according to ALL-BFM 2000 (NCT 00430118) and AIEOP-BFM-ALL 2009 treatment protocols (NCT 01117441). THROMBOTECT was an add on-study to the ALL-BFM protocols and approved by the leading ethics committees of the Medical School Hannover, Germany, and St. Gallen, Switzerland, and by the local ethics committees of each participating site. Written informed consent was obtained from guardians and/or patients before randomization. The detailed study protocol is available online with the supplemental material.

Patient eligibility

Patients were eligible if treated on the ALL-BFM 2000 or AIEOP-BFM ALL 2009 protocol[28-30] and having a CVC inserted by day 8 of induction and remaining in place at least until day 33. The choice of the CVC and its maintenance was at the treating physician's discretion according to institutional guidelines. In August 2004, the protocol was amended to allow participation of patients without CVC. Exclusion criteria were known hemorrhagic disorders unrelated to leukemia, active gastrointestinal ulcer, previous cerebrovascular accident and/or known hypersensitivity to heparin.

Randomization and Study Treatment

After written informed consent had been given, randomization was performed by day 8 in a 1:1:1 ratio using permuted blocks of 6 patients and stratified by country and the glucocorticoid preparation (dexamethasone or prednisone) administered during induction.[29] Randomization was centrally performed by the ALL-BFM study coordination center using computer-generated random number lists. This ensured that the participating centers had no access to the allocation sequence. The assigned arm was submitted to the centre by fax.

Patients were randomly assigned to receive one of the two experimental thromboprophylactic treatments with either LMWH enoxaparin or with activity-adjusted antithrombin or to the control arm low-dose unfractionated heparin (UFH).

Thromboprophylaxis was started on day 8 and ended on day 33 of induction chemotherapy (Figure S1 in the Supplementary Appendix). The observation period covered the induction and consolidation phase (Figure S2 in the Supplementary Appendix) up to and including protocol day 64.

Patients in the enoxaparin group received Clexane™ at 80-100 IU/kg body weight once daily subcutaneously[31-34] with a target anti-Xa level not exceeding 0.4 U/l, measured 4 hours after the third or fourth injection. On days with lumbar puncture or other invasive procedures, enoxaparin was postponed until at least 4 hours after the procedure. In the case of

thrombocytopenia <30 x 10^9/L, platelet tranfusion was required or enoxaparin had to be held until platelet regeneration.

In the antithrombin group, antithrombin activity was measured every three days prior to each asparaginase administration. If activity was below the lower limit of normal of 80%, the plasma-derived antithrombin preparation Kybernin™ was substituted calculating the dose according to the formula [$antithrombin_{target\ 100\%}$ – $antithrombin_{actual}$] x kg body weight targeting at 100% AT activity.

Patients assigned to the control arm received UFH at 2 IU/kg body weight/hour as long as an infusion drip was running to locally prevent thrombotic occlusion of the indwelling CVC.[24]

Treatment with coagulation factors or anticoagulants beyond the interventions intended per protocol was not allowed unless clinically indicated. Management of TE was at the discretion of the treating physician.

Outcome Measures

Diagnosis of TE was based upon clinical suspicion and had to be confirmed by one or more suitable imaging methods within routine diagnostic work-up (Table S1 in the Supplementary Appendix). No systematic provision was made for blinding the attending physicians or radiologists to the randomization arm. Intermittent dysfunction of the CVC by a clot at the tip of the catheter was not considered a thrombotic event as long as CVC patency was restored.

The principal safety outcome was absence of bleeding complications during the study period. The definition of major and minor hemorrhage met internationally defined standards (Table S2 in the Supplementary Appendix).[35-37] Secondary safety outcomes were event-free survival (EFS) and overall survival (OS). EFS was defined as time from diagnosis to the date of last follow-up or first event. Events were resistance to therapy, leukemia relapse, secondary neoplasm or death from any cause. Failure to achieve remission due to early death or resistance was considered as event at time zero. Survival was defined as time from diagnosis to the date of last follow-up or death from any cause.

Statistical Analysis

The primary objective was to test whether antithrombotic prophylaxis with enoxaparin or antithrombin was superior to UFH. The null hypothesis was that there was no difference between enoxaparin or antithrombin versus UFH tested with one-tailed Fisher's exact test at a significance level of P=0.025 each. The main analysis was by intention-to-treat (ITT). In order to reach a power of 85% with a significance level of 0.025, 315 patients had to be randomized per group, assuming an event rate of 9% within the UFH group and 3% in the two interventional groups, respectively. If both comparisons were significantly different, the thrombosis rates in the enoxaparin and antithrombin arm had to be tested for equivalence (secondary objective). Antithrombin replacement and enoxaparin therapy would be considered equivalent if the two-sided 95% confidence interval (95%-CI) of the incidence difference did not exceed ±4%. For the equivalence test, patients were analyzed according to the given treatment (as treated).

The Kaplan-Meier method[38] was used to estimate survival rates, and differences were compared with the log-rank test.[39] Cox proportional hazards model was used in univariate and multivariate survival analyses.[40] Cumulative incidence functions for competing events were constructed by the method of Kalbfleisch and Prentice[41] and compared with the Gray's test.[42] Odds ratios were calculated to compare the risks of thromboembolic events. Except for the confirmative analyses of the primary study question, all other analyses were exploratory.

Results

Patient Characteristics

From December 1st, 2002, to December 31st, 2011, 1526 patients with ALL treated at one of the 26 study centers in Germany and Switzerland were eligible for randomization (Figure 1). Of these, 577 patients were not randomized, the vast majority because patients and/or parents refused consent to be randomized for the enoxaparin arm as they strictly did not wish to accept a daily subcutaneous injection. 949 patients (ITT population) were randomly

assigned to receive either UFH (N=312), enoxaparin (N=317) or antithrombin (N=320). Randomized and non-randomized eligible patients did not differ with respect to their initial patient characteristics (Table S3 in the Supplementary Appendix). The proportion of patients with poor response to the prednisone prephase (prednisone poor-responders) and slow treatment response as assessed by minimal residual disease was significantly higher in the group of non-randomized patients. In the ITT population, numbers and characteristics of patients were well balanced between the three randomization arms except for a slight imbalance in the age distribution with fewer children below six years in the enoxaparin group (Table 1). Patient characteristics were evenly distributed between the randomization arms as treated except for a significantly lower proportion of patients below 6 years of age in the enoxaparin arm (details provided in Table S4 in the Supplementary Appendix).

The proportion of patients who refused antithrombotic treatment as allocated was 3% in patients randomized to UFH (10/312) or antithrombin (11/320), and 33% (105/317) in those assigned to enoxaparin (Figure 1). Rejection of the enoxaparin arm was more frequent in patients below six years of age than in older patients (62/157 [39%] vs. 42/160 [27%]) with a preferential switch to UFH in the younger cohort (Table S5 in the Supplementary Appendix). Based on this finding additional exploratory analyses with respect to TE rate and leukemia-related outcomes were therefore performed, stratified by age and in the as-treated groups.

Thromboembolic Events

Among the 949 randomized patients, 42 thromboembolic events were observed (4.4%; 95%-CI 3.2 to 5.9). Of those, 20 events (47.6%) occurred in the upper, seven (16.7) in the lower deep venous system, and 13 (30.9%) in the cerebral sinus veins; two patients (4.8%) had a cerebral arterial stroke. Eight of the 42 TEE (19%) were distant to the site of the CVC. Thirty-three events occurred between treatment day 9 and 36 during induction therapy, nine events between treatment day 37 and 52 of induction consolidation.

Children below six years of age had a significantly lower risk of TE (14/512, 2.7%) than those aged 6 to 9 years (11/188, 5.9%) or 10 years and older (17/249, 6.8%; P=0.018). Other patient characteristics and features, such as gender, initial white blood cell count,

immunophenotype or treatment response did not influence the incidence of TE (data not shown).

The incidence of TE was significantly higher in patients randomized to UFH (25/312; 8.0%) than in the enoxaparin (11/317; 3.5%; P=0.011) or antithrombin group (6/320; 1.9%; P<0.001). The as-treated analysis revealed an incidence of 6.7% in the UFH group (25/372) compared to 3.2% in the enoxaparin (7/216; P=0.089) and 2.6% in the antithrombin group (9/341; P=0.013). The respective cumulative incidences are depicted in Figures 2A and B. The difference between TE incidences in the enoxaparin and antithrombin group as treated was -0.6%; the lower and upper limit of the 95%-CI were -3.5% and +2.3%, respectively (p-values for the corresponding one sided tests P=0.01 and P=0.001). Thus, antithrombin and enoxaparin were equally effective.

Exploratory as-treated analyses stratified by age (Figures 2D and F) demonstrated a significantly reduced risk of TE in patients six years of age or older when treated in one of the experimental arms compared to the control group (UFH: 18/158, 11.4%; enoxaparin: 5/120, 4.2%, P(vs. UFH)=0.001; antithrombin 4/150, 2.7%, P(vs. UFH)<0.001). No significant differences were found in patients below six years of age (UFH 7/214, 3.3%; enoxaparin 2/96, 2.1%; antithrombin 5/191, 2.6%).

For subgroup analysis by age no formal test for interaction was done. Applying Fine-Gray models with interaction terms for age older than 6 years and enoxaparin/antithrombin, the interactions are not significant. This, however, does not entirely exclude interactions since the power for such tests is low.

Hemorrhage

Eight bleeding episodes were documented among the 929 randomized patients (0.9%). Four of them occurred during induction chemotherapy under antithrombotic prophylaxis and 4 during consolidation after termination of the anticoagulants. All hemorrhages were classified as major (7 gastrointestinal, 1 cerebral). Four patients with hemorrhage were treated in the UFH group (1.1%), three in the antithrombin group (0.9%, P(vs. UFH)=1.0) and one patient in the enoxaparin group (0.5%, P(vs. UFH)=0.66).

Leukemia Outcome, Survival

Five-year probability of EFS (5y-pEFS) and cumulative incidence of relapse (5y-CIR) of the THROMBOTECT cohort were comparable with the 577 non-randomized patients (THROMBOTECT cohort: 5y-pEFS 84.3±1.2%, 5y-CIR 11.7±1.1%; non-randomized patients: 5y-pEFS 84.0±1.6%, 5y-CIR 11.8±1.4). Patients randomized to the antithrombin arm had a 5y-pEFS of 80.9±2.2% compared with those assigned to the enoxaparin (86.2±2.0%, P=0.10) or UFH arm (85.9±2.0%, P=0.06) (Figure 3A) with a Hazard ratio of 1.40 (1.02-1.92; P=0.040) for the antithrombin arm versus the remaining patients. The probability of OS at 5 years was similar in all three arms (antithrombin 89.8±1.7%, enoxaparin 90.9±1.6%, UFH 92.4±1.5%). The differences observed in the EFS were due to a higher incidence of late relapses in the antithrombin group as compared to the other groups (Figure 3C); the as-treated analyses showed no statistically significant difference between the three groups (Figure 3B and D; Hazard ratio antithombin vs. others: 1.16 [0.84-1.59]; P=0.37). Retrospective exploratory subgroup analyses revealed a higher relapse incidence of the antithrombin-treated patients within the medium risk group only (Figure S3 in the Supplementary Appendix). Multivariate Cox regression analyses on EFS were performed including risk group according to respective trial criteria, *TEL-AML1* status, initial white blood cell count, age and the THROMBOTECT arm as covariates. Hazard ratios for the antithrombin arm were 1.38 (0.99-1.91; P=0.054) for ITT and 1.19 (0.86-1.66; P=0.269) for the as-treated analysis and thus comparable with those of the univariate analyses (Table S6 in the Supplementary Appendix).

To test for a potential dose effect of antithrombin, doses given were analyzed in patients treated in the antithrombin arm. Data available for 248 of 341 patients (72.7%) did not disclose a dose-related effect on the relapse incidence (Figure S4 in the Supplementary Appendix).

Discussion

Reliable data on TE during induction therapy of childhood ALL are scarce. The only randomized interventional trial was the PARKAA trial (Prophylactic Antithrombin replacement in kids with ALL treated with L-asparaginase), designed to determine if there was a trend to efficacy and safety of antithrombin treatment but not powered to prove it.[16] To our knowledge, no other data from adequately designed and powered studies have been available so far to provide sufficient evidence that would allow valid recommendations.[4,5,9,19,20,23,43,44]

For the first time, the THROMBOTECT trial shows that prophylactic antithrombotic intervention significantly reduced TE during ALL induction therapy as compared to the control arm. Both interventions, enoxaparin and activity adapted AT substitution, were equally effective. Asparaginase induced AT deficiency is assumed to be the most important mechanism for the development of TE during ALL induction therapy.[45] As a consequence of asparagine depletion, asparaginase therapy leads to intracellular retention of a misfolded antithrombin, resulting in acquired antithrombin deficiency.[45,46] The THROMBOTECT trial demonstrated that maintaining the AT activity at 80% or higher throughout the induction phase could significantly protect patients from TE. Thus, correction of low antithrombin activity seems to be one effective way to prevent TE, this being consistent with clinical and laboratory data on antithrombin supplementation.[10,16,18,19,47]

A considerable number of patients eligible for the study were not randomized. In this group the rate of prednisone poor-responders was significantly higher than in the THROMBOTECT cohort. This may be attributed to a tendency of the doctors or parents to avoid additional burden from interventions of an add-on trial in particular on those patients with very poor response during the first days of treatment. However, patient characteristics were comparable between the three randomization groups except for a slight underrepresentation of younger patients assigned to enoxaparin. Yet, the main reason not to participate was the refusal to accept the daily subcutaneous enoxaparin injections. Not surprisingly, the proportion of patients and parents refusing the assigned enoxaparin was highest in young children. This demonstrates not only their reluctance to receive injections but also underlines

a considerable drawback in practical use, irrespective of the antithrombotic efficacy of enoxaparin.

Older age proved to be an important risk factor for TE as it has been reported earlier by others.[1,13,48] The best cut-off in our data was the age of six years. Exploratory analyses suggested that the benefit from either experimental arm was more pronounced in older patients than in young children. The significant benefit in risk reduction of TE with either intervention, enoxaparin or antithrombin, as compared to UFH, provides a convincing rationale for thromboprophylaxis in this age group. For younger children, the incidence of TE was low and comparable in all three randomization arms. The need of a thromboprophylaxis in ALL patients below 6 years of age could therefore be questioned. However, the study was not powered for subgroup analyses and the lack of statistical difference in TE incidence between the treatment groups in younger children may be due to insufficient power caused by the patient number as well as the lower TE incidence. Furthermore, in younger children TE may be missed as symptoms often are subtle. This is in line with the findings of the PARKAA study, showing that children with symptomatic TE tend to be older than those with clinically asymptomatic TE.[16] Even if clinically not diagnosed, asymptomatic TE may be associated with significant vessel occlusion.[16] This, in turn, can lead to the destruction of the vessel wall causing long term morbidity in terms of postthrombotic syndrome, likely becoming apparent years after the end of ALL therapy. Whether this applies to young patients with ALL remains unknown.[17] Future studies with sufficient statistical power are needed to ascertain if such interventions in small children are justified. Nevertheless, although the high proportion of patients who refused the allocation to the enoxaparin arm may complicate the interpretation of the results in this treatment arm, the reduction of TE in the global analysis appears to be sufficiently convincing to recommend thromboprophylaxis not only for older patients but for all age groups, all the more as hemorrhage is of no concern.

Most thrombotic events occurred between induction treatment day 9 and 36, the latter marking the start of induction consolidation. This confirms our experience that TE only rarely occurs at the time of ALL diagnosis but rather in the course of induction therapy.

Furthermore, not all centers were able to get a CVC inserted at the time of ALL diagnosis. For these reasons, thromboprophylaxis was started after the prednisone prephase on day 8 of induction therapy. The primary objective of the THROMBOTECT trial was to evaluate efficacy and safety of different prophylactic antithrombotic interventions during ALL induction therapy. Therefore, the duration of thromboprophylaxis was limited to induction therapy until day 33. Some of the thromboembolic events have occurred after the end of the induction phase. However, only a few of these patients had already started the consolidation phase when the thrombosis had been diagnosed. Factors that may have contributed to these late thromboses could be concurrent medical issues such as infections. Given the gradual development of a clot, the still asymptomatic thrombosis might have started towards the end of induction therapy and only become symptomatic in early induction consolidation. Since pegylated asparaginase is presently used more frequently - in the trial AIEOP-BFM ALL 2009, the second dose of this drug was given on day 26 of induction - late thromboses in induction consolidation might become more relevant as the use of pegylated asparaginase may lead to an extended asparagine depletion with disturbed coagulation patterns, including extended dysfunction of antithrombin. Irrespective of possible concomitant prothrombotic risk situations, the hypercoagulable state seems to remain ongoing beyond the end of induction therapy. Given the very low rate of hemorrhage it might therefore be advisable to extend thromboprophylaxis accordingly.

The open label assignement as well as the diagnosis of TE on clinical suspicion only are drawbacks of the THROMBOTECT study design. However, masking the antithrombotic intervention would have meant that all patients of all randomization groups would have been given subcutaneous injections, in the UFH and AT group containing placebo. To conduct the study as double-blinded trial with double dummy subcutaneous injections was not considered feasible in a large pediatric population.

Similar concerns apply to the primary outcome defined as TE based on clinical suspicion. The PARKAA study has shown a high incidence of clinically not recognized thromboses found by routine imaging screening.[16] To overcome observer bias, various and repeated

routine imaging screening for vessel occlusion at all possible anatomical sites would have been mandatory at predefined time points. This comprises ultrasound but also magnetic resonance imaging which, in young children, often requires general anaesthesia. In addition, for the time being the appropriate time points to look for vessel occlusions is not known and hence the possibility of missing a thrombosis at arbitrarily chosen time points would be high. Exposing the children to repeated extra anaesthesia with a questionable benefit was considered too high an extra burden. Therefore, the study design chosen was in favour of an open label treatment. Imaging was performed on clinical suspicion despite the acknowledged inherent drawbacks.

Evaluation of EFS and relapse rate within the THROMBOTECT randomization groups revealed the unexpected finding that patients randomized to the antithrombin group had a higher relapse incidence compared with the enoxaparin or UFH group. The differences were no longer obvious in the as-treated analysis being apparent in the medium risk group only. Although a causal relationship between the cumulative antithrombin dose and the relapse rate could not be established, the possibility that antithrombin substitution might affect leukemia outcome cannot be entirely excluded.

In conclusion, the THROMBOTECT study has for the first time demonstrated that activity-targeted antithrombin replacement as well as the use of enoxaparin lead to a significant risk reduction for TE during ALL induction therapy when compared with low-dose UFH. Bleeding was of no major concern. Thromboprophylaxis during induction therapy can therefore be recommended for children and adolescents with ALL. The higher incidence of late relapses in children with medium risk ALL assigned to the antithrombin group remains to be resolved and leads us at the present time to recommend primarily enoxaparin. Whether thromboprophylaxis contributes to minimize not only clinical but also silent thrombosis and by that long term morbidity in terms of postthrombotic syndrome remains to be determined. The THROMBOTECT results provide the rationale to develop new studies, both to elucidate a possible impact of antithrombin on leukemia outcome and to further determine the best practice to prevent TE during ALL induction chemotherapy. *3902 words (main text)*

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Author's disclosures of potential conflicts of interest

Both interventional drugs were provided free of charge by the respective pharmaceutical companies: Enoxaparin (Clexane™) by Sanofi and antithrombin (Kybernin™) by CSL Behring. Neither of the two companies was acting as a sponsor, they were not involved in the THROMBOTECT study design, neither in the collection and analysis of data nor in the content and wording of the manuscript. Neither of them had access to the THROMBOTECT data sets nor did they have information on unpublished results.

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Table 1. Patient characteristics by thromboprophylaxis group as assigned by randomization

	total (N=949) N (%)	UFH (N=312) N (%)	E (N=317) N (%)	AT (N=320) N (%)
Study				
ALL-BFM 2000	815 (85.9)	269 (86.2)	272 (85.8)	274 (85.6)
AIEOP-BFM ALL 2009	134 (14.1)	43 (13.8)	45 (14.2)	44 (13.8)
Sex				
Male	537 (56.6)	173 (55.4)	183 (57.7)	181 (56.6)
Female	412 (43.4)	139 (44.6)	133 (42.3)	139 (43.4)
Age				
1 – < 6 years	512 (54.0)	174 (55.8)	157 (49.5)	181 (56.6)
6 – < 10 years	188 (19.8)	57 (18.3)	72 (22.9)	59 (18.4)
≥ 10 years	249 (26.2)	81 (26.0)	88 (27.8)	80 (25.0)
Central venous catheter				
CVC in site	896 (94.4)	295 (94.6)	294 (93.3)	303 (95.2)
No CVC	53 (5.6)	17 (5.4)	21 (6.7)	15 (4.8)
WBC at diagnosis [x10^9/L]				
< 20	599 (63.1)	199 (63.8)	212 (66.9)	188 (58.8)
20 - < 100	249 (26.2)	83 (26.6)	76 (24.0)	90 (28.1)
100 - < 200	53 (5.6)	15 (4.8)	14 (4.4)	24 (7.4)
≥ 200	47 (5.0)	15 (4.8)	14 (4.4)	18 (5.6)
CNS status				
CNS negative	872 (91.9)	278 (89.1)	298 (94.0)	296 (92.5)
CNS positive	30 (3.2)	14 (4.4)	6 (1.9)	10 (3.1)
no information	47 (5.0)	20 (6.4)	13 (4.1)	14 (4.4)
Immunophenotype				
Non-T-ALL	827 (87.1)	264 (84.6)	298 (89.0)	281 (87.8)
T-ALL	120 (12.6)	47 (15.1)	34 (10.7)	39 (12.3)
no information	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)
Genetics				
t(12;21) / *TEL-AML1*				
negative	722 (76.1)	235 (75.3)	245 (77.3)	242 (75.6)
positive	199 (21.0)	65 (20.8)	63 (19.9)	71 (22.2)
no information	28 (3.0)	12 (3.8)	9 (2.8)	7 (2.2)
t(9;22) / *BCR-ABL*				
negative	924 (97.4)	303 (97.1)	309 (97.5)	312 (97.5)
positive	25 (2.6)	9 (2.9)	8 (2.5)	8 (2.5)
no information	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

	total (N=949) N (%)	UFH (N=312) N (%)	E (N=317) N (%)	AT (N=320) N (%)
t(4;11) / *MLL-AF4*				
negative	942 (99.3)	311 (99.7)	314 (99.1)	317 (99.1)
positive	7 (0.7)	1 (0.3)	3 (0.9)	3 (0.9)
no information	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Peripheral blast count on day 8 (Prednisone Response)				
< $1x10^9$/L (PGR)	880 (92.7)	291 (93.3)	295 (93.1)	294 (91.9)
≥ $1x10^9$/L (PPR)	65 (6.8)	19 (6.1)	22 (6.9)	24 (7.5)
no information	4 (0.4)	2 (0.6)	0 (0.0)	2 (0.6)
Risk group				
SR	301 (31.7)	97 (31.1)	101 (32.1)	101 (31.8)
MR	512 (54.0)	171 (54.8)	169 (53.7)	170 (53.5)
HR	136 (14.3)	44 (14.1)	45 (14.3)	47 (14.8)
MRD at end of induction				
negative	303 (31.9)	103 (33.0)	104 (32.8)	96 (30.0)
< 5×10^{-4}	316 (33.3)	107 (34.2)	113 (35.6)	96 (30.0)
≥ 5×10^{-3}	184 (19.4)	57 (18.3)	58 (18.3)	69 (21.6)
no information	146 (15.4)	45 (14.4)	42 (13.2)	59 (18.4)
MRD at week 12				
negative	579 (61.0)	187 (59.9)	202 (63.7)	190 (59.4)
< 5×10^{-4}	146 (15.4)	53 (17.0)	47 (14.8)	46 (14.4)
≥ 5×10^{-3}	43 (4.5)	16 (5.1)	12 (3.8)	15 (4.7)
no information	181 (19.1)	56 (17.9)	56 (17.7)	69 (21.6)
Randomized in induction in AIEOP-BFM ALL 2000*				
Randomized				
assigned to PDN	125 (13.2)	39 (12.5)	41 (12.9)	45 (14.1)
assigned to DXM	136 (14.3)	45 (14.4)	45 (14.2)	46 (14.4)
Not randomized	688 (72.5)	228 (73.1)	231 (72.9)	229 (71.6)

*For details see Figure S2 in the Supplementary Appendix and Reference Möricke, Blood (2016).[19]

Abbreviations: AT, antithrombin; CNS, central nervous system; CVC, central venous catheter; DXM, dexamethasone; E, enoxaparin; HR, high risk, MR, medium risk; MRD, minimal residual disease; PDN, prednisone; PGR, Prednisone Good-Response; PPR, Prednisone Poor-Response; SR, standard risk; UFH, unfractionated heparin; WBC, white blood cell count.

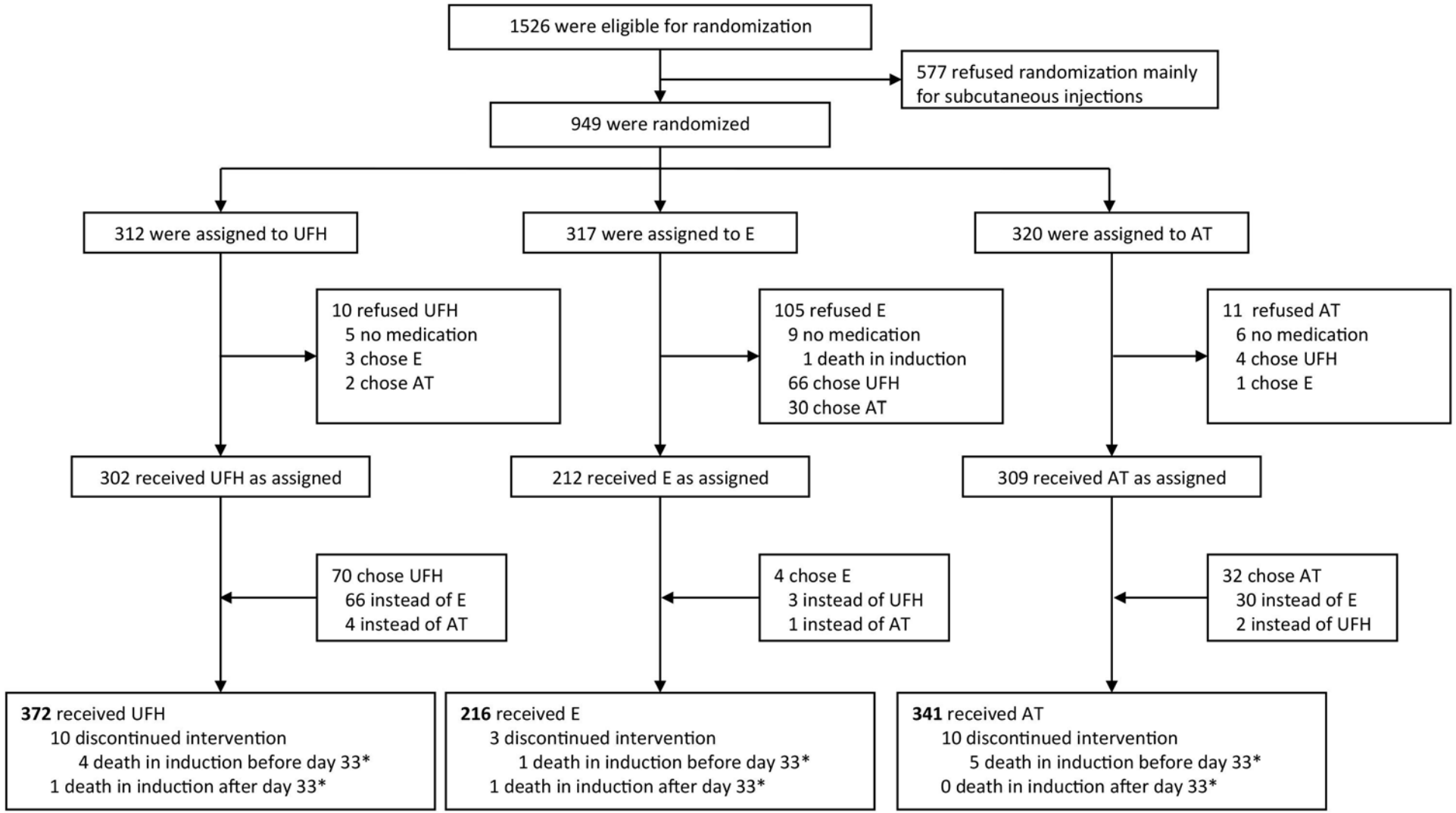
Figure legends:

Figure 1: Consolidated Standards for Reporting of Trials (CONSORT) diagram. AT denotes antithrombin, E denotes enoxaparin, UFH denotes unfractionated heparin.

Figure 2: Thromboembolic events according to the randomization arms. Results are shown by intention to treat (A, C and E) and by treatment as given (B, D and F) for the total cohort (A and B) and stratified by age < 6 years (C and D) and ≥ 6 years (E and F). Events are depicted as cumulative incidence curves. Indicated P values were calculated with the Fisher's exact test. TEE denotes thromboembolic event; UFH denotes unfractionated heparin; OR denotes Odds ratio; CI denotes confidence interval.
AT denotes antithrombin, E denotes Enoxaparin, TEE denotes thromboembolic events, UFH denotes unfractionated heparin.

Figure 3: Outcome of ALL according to the THROMBOTECT randomization arms. Event-free survival (A and B) and cumulative incidence of relapse (C and D) are shown by intention to treat (A and C) and by treatment as given (B and D). Numbers of patients at risk in the event-free survival graphs also apply to the respective relapse incidence graphs. 5 y-pEFS denotes 5-year probability of event-free survival; 5 y-CIR denotes 5-year cumulative incidence of relapse; SE denotes standard error; UFH denotes unfractionated heparin.

Figure 1



* Day 33: end of interventional treatment phase

Figure 2A

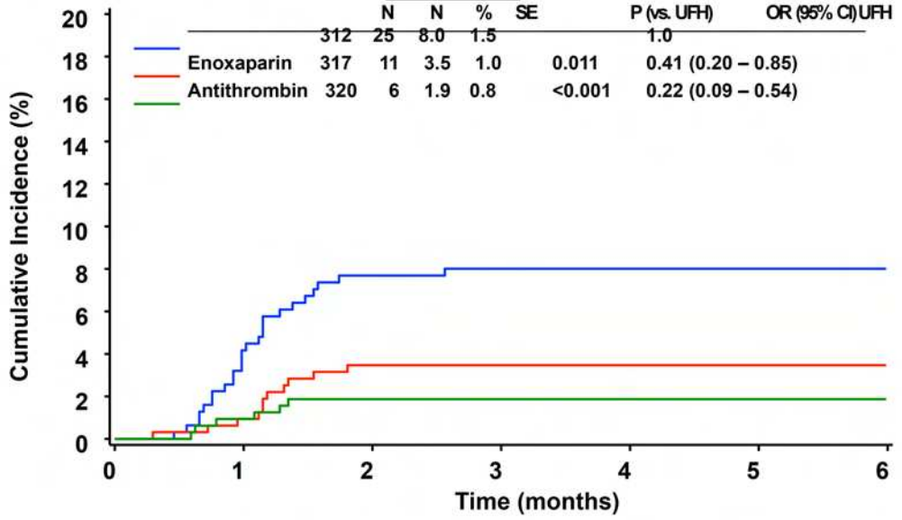


Figure 2B

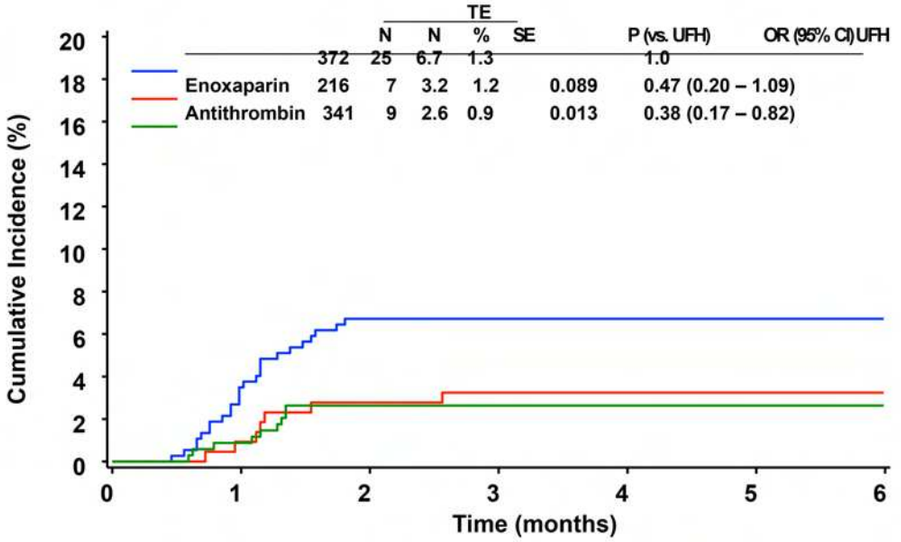


Figure 2C

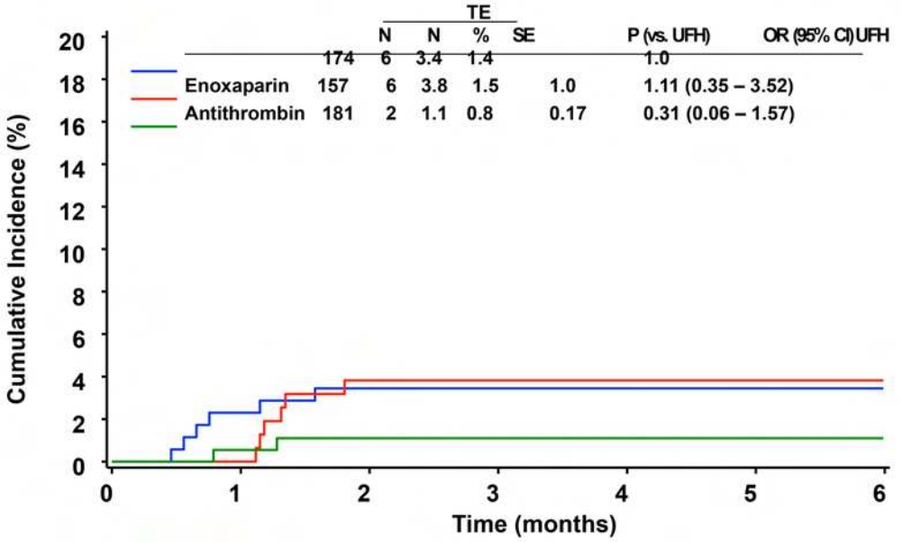


Figure 2D

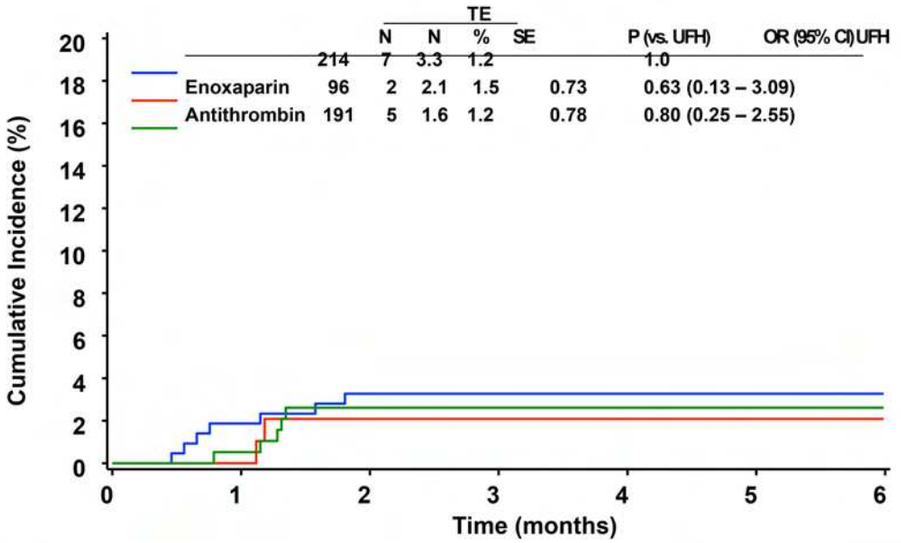
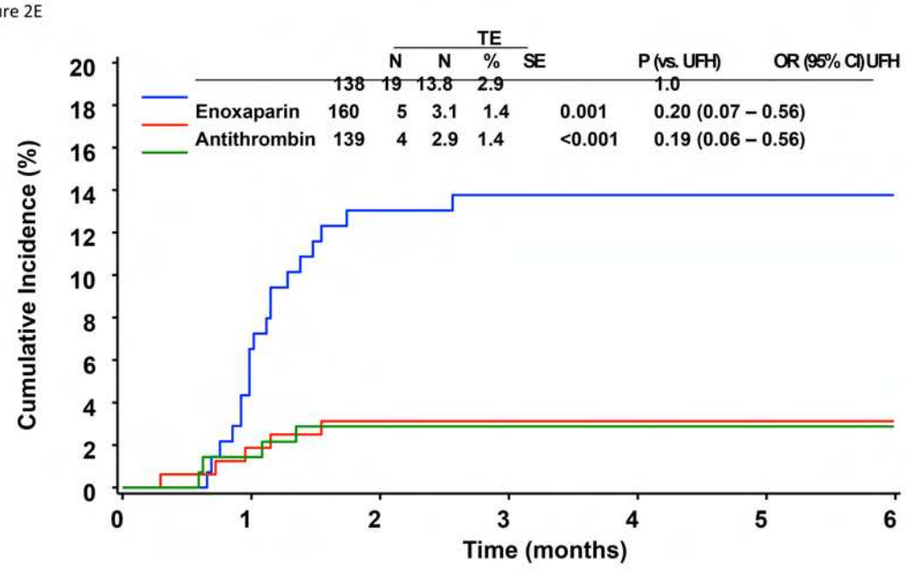


Figure 2E



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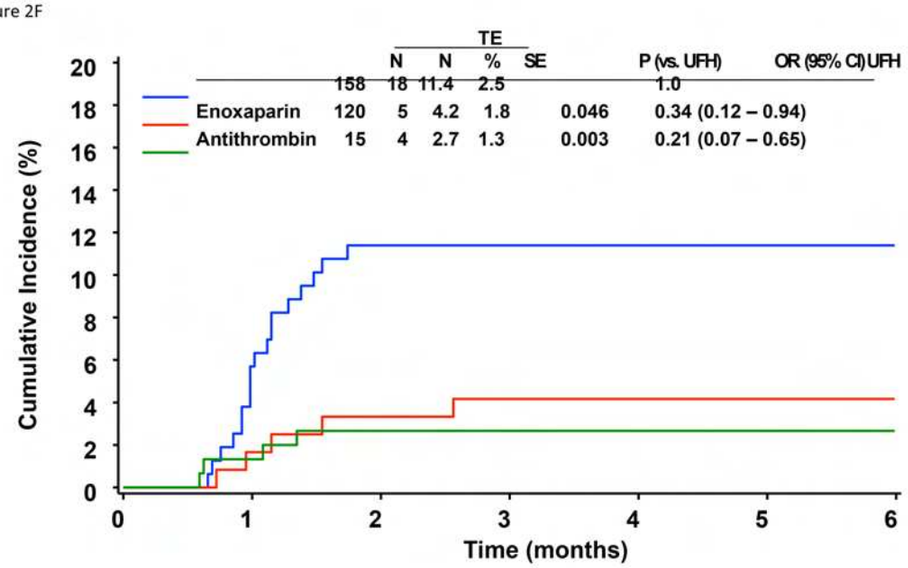
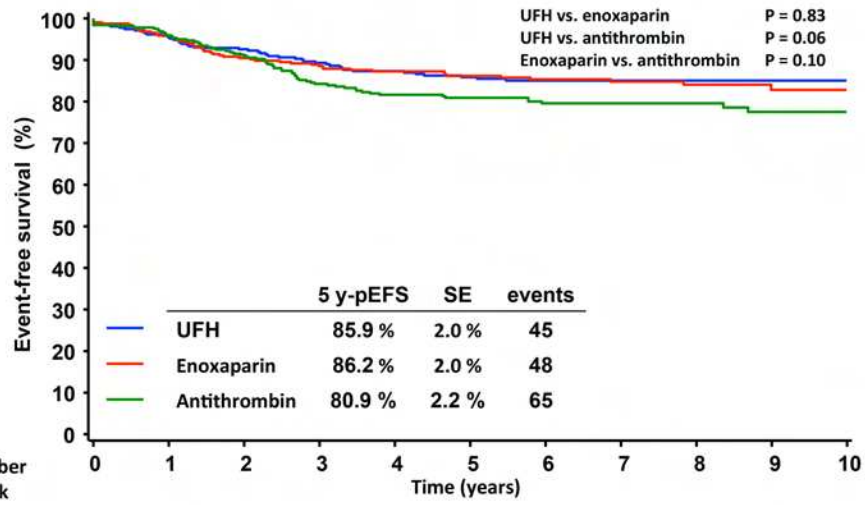
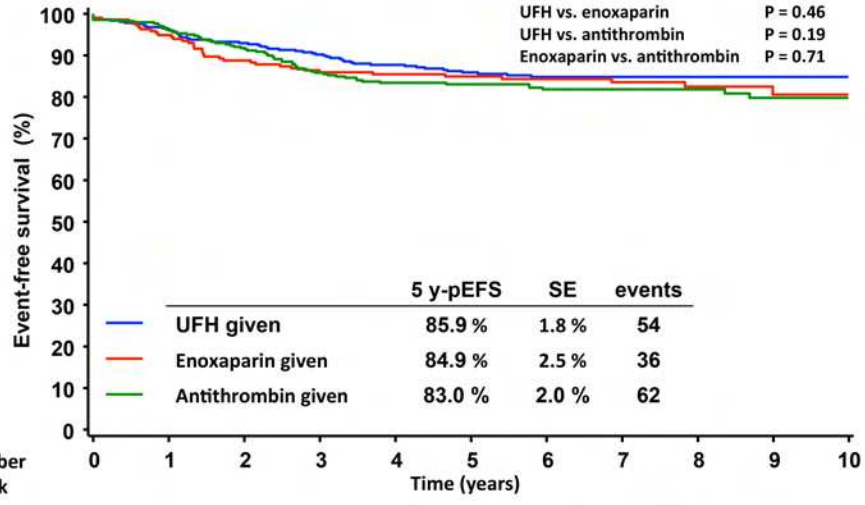


Figure 3A



Number at risk										
UFH	312	296	284	270	255	215	181	152	101	65
Enoxaparin	317	302	283	275	261	228	185	151	106	67
Antithrombin	320	306	290	264	245	213	174	124	92	64

Figure 3B



Number at risk										
UFH	372	356	340	326	307	262	214	180	122	86
Enoxaparin	216	203	189	181	175	151	127	103	73	42
Antithrombin	341	327	311	287	265	232	190	137	97	66

Figure 3C

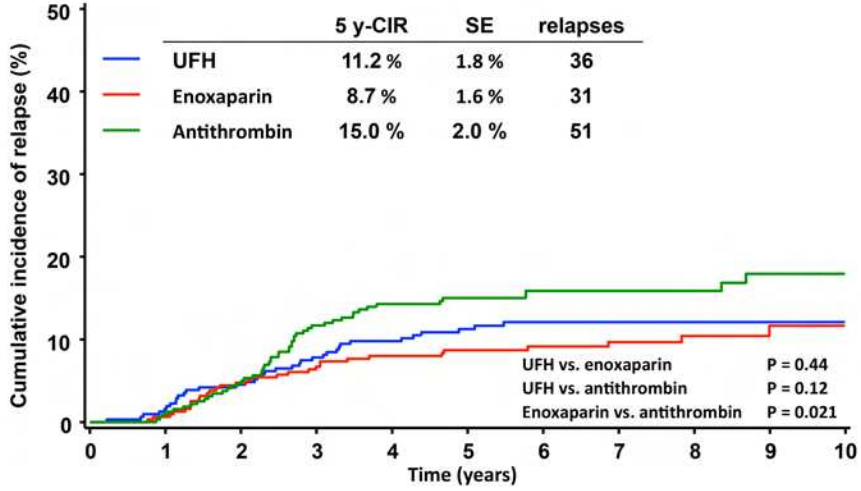


Figure 3D